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(19) (CA) APPLICATION FOR CANADIAN PATENT (12)

(54) Peptides Having Neuroprotective and Immunostimulatory Functions

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PEPTIDES HAVING NEUROPROTECTIVE AND IMMUNOSTIMULATORY FUNCTIONS

ABSTRACT OF THE DISCLOSURE

Histogranin having the structure H-Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH and analogs thereof having NMDA and/or AMPA receptor agonist and/or antagonist activities both *in vivo* by their blockade of NMDA- and AMPA-induced convulsions and *in vitro* by their binding and allosteric interactions with domains of the NMDA receptor complex and their potentiation of host-defense immune responses (IL-1 production and IL-1α mRNA induction in alveolar macrophages) are herein described. Histogranin is a natural compound which can be extracted from suitable tissue, e.g. bovine adrenal medulla, or synthesized using the solid-phase peptide synthesis procedure. Histogranin and specific active fragments and analogs are potent ligands of a specific receptor linked to the NMDA and/or AMPA receptors: they block NMDA- and AMPA-receptor stimulation and therefore can be considered as good protecting agents against nerve cell degeneration due to an overstimulation of the NMDA and/or AMPA receptors, an event observed in the following brain disturbances: ischaemia, stroke, epilepsy, Alzheimer's disease, spinal cord trauma, anxiety, schizophrenia and Huntington's disease. Some analogs are also proposed as putative antagonists of the histogranin receptor and therefore have potential beneficial use as enhancer of NMDA receptor stimulation, a process involved in learning and memory. In periphery, the ability of histogranin (and analogs) in enhancing the immune responses of immune cells such as alveolar macrophages stresses its potential use for the treatment of immune disorders (immunodeficiency related to AIDS, U.V. exposure or other immune disorders).

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates to peptides having neuroprotective and immunostimmulatory functions, to the extraction or synthesis of such peptides, and to the uses of such peptides for the management of neurodegenerative and/or immune diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Since reference is made in the immediately following description to the accompanying drawings, the figures shown in the drawings are first briefly described as follows.

Figure 1 is a schematic representation of the NMDA receptor complex, its stimulation, its regula. .n and its overstimulation and resulting cytotoxic effect on stimulated neuron; a hypothetical binding site proposed by the inventor for the negative allosteric regulator, histogranin [SEQ ID NO:1], is shown on this receptor, but this remains to be proven.

Figure 2(A) is an analytical high performance liquid chromatogram (HPLC) of adrenomedullary histogranin [SEQ ID NO:1] on an SP-5PW column (Waters Inc).

Figure 2(B) is an analytical HPLC of adrenomedullary histogranin [SEQ ID NO:1] on a Zorbax ODS column (Trademark, Dupont).

Figure 3 is an analytical HPLC on Zorbax ODS column (Trademark, Dupont) of synthetic [Ser^1]histogranin [SEQ ID NO:2] (50 pmol). Inset: fast atom bombardment (FAB) mass spectrometry of the synthetic peptide.

Figure 4(A) is an analytical HPLC on a μ-Bondapak C18 column (Trademark, Waters) of immunoreactive (ir)-histogranin (HN) [SEQ ID NO:1] present in an extract of secretory granules isolated from bovine adrenal medulla, the arrow indicating the retention time of synthetic HN [SEQ ID NO:1].

Figure 4(B) is an analytical HPLC on a μ-Bondapak C18 column of adrenal perfusates before and after stimulation with carbamylcholine (CCh, 5 x 10^{-4} M); the arrow indicating the retention time of synthetic HN [SEQ ID NO:1].

Figure 5 is an autoradiogram of thin layer sections of bovine adrenal glands pre-incubated with [^{125}I]-[Ser^1]HN [SEQ ID

NO:2]in presence (B) or absence (A) of unlabelled HN [SEQ ID NO:1] (20 μM).

Figures 6(A) and (B) are graphs showing the effect of HN [SEQ ID NO:1] on the binding of [^{3}H]dextromethorphan (5 nM) to membrane preparations of bovine adrenal medulla (A) and rat brain (B).

Figure 7 is a graph showing the binding of [^{125}I][Ser^1]histogranin [SEQ ID NO:2] to rat brain membranes in function of the concentration of membrane protein.

Figure 8 is a graph showing the saturation isotherm of [^{125}I][Ser^1]histogranin [SEQ ID NO:2] binding to rat brain membranes. Inset: Scatchard plot analysis of the data.

Figure 9 is a graph showing the association and dissociation of [^{125}I][Ser^1]histogranin [SEQ ID NO:2] binding to rat brain membranes.

Figure 10(A) is a graph showing the effect of increasing concentrations of histogranin (HN) [SEQ ID NO:1] and [Ser^1] histogranin ([Ser^1]HN) [SEQ ID NO:2] on the specific binding of [^{3}H]dextromethorphan ([^{3}H]DM) to rat brain membrane preparations.

Figure 10(B) is a graph showing the effect of (+)pentazocine (1 μM), MK-801 (1 μM), NMDA (100 μM) and CPP (1 μM) on the potentiation of [^{3}H]DM binding by increasing concentrations of [Ser^1]HN [SEQ ID NO:2].

Figure 11 is a graph showing the potentiation of the secretion of interleukin 1 (IL-1) from cultured rat alveolar macrophages in response to lipopolysaccharide (LPS; 5 μg/ml) by increasing concentrations of [Ser^1]histogranin ([Ser^1]HN) [SEQ ID NO:2].

Figure 12 represents the potentiation of the production of interleukin 1α (IL-1α) mRNA by increasing concentrations of [Ser^1]HN [SEQ ID NO:2]in cultured rat alveolar macrophages challenged with LPS (C: control, HN: [Ser^1]HN) [SEQ ID NO:2].

Figure 13 shows the modulatory effects of increasing concentrations of histogranin on the binding of specific ligands of NMDA ([^{3}H]CGP 39653), PCT ([^{3}H]MK-801) and σ ([^{3}H](+)-pentazocine) to membrane preparations of rat brain.

The curves are representative of four replicate experiments (means ± S.E.), • = [^{3}H]CGP 39653; o = [^{3}H]MK-801; ■ = [^{3}H](+)pentazocine.

DESCRIPTION OF THE PRIOR ART

Excitatory amino acids such as L-glutamate (Glu) and L-aspartate (Asp), are major neurotransmitters in the mammalian central nervous system (CNS). Multiple acidic amino acid receptor subtypes exist for these neurotransmitters. For example, these include ion channel-linked receptors mediating neuronal depolarization, named after the protypical agonists N-methyl-D-aspartate (NMDA), alpha-amino-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and a putative presynaptic stimulator, L-2-amino-4-phosphonobutyrate (L-AP4). A fifth excitatory amino acid receptor is the metabotropic receptor, linked to phosphoinositide metabolism (Farooqui and Horrocks, Brain Res. Rev. 16, 171, 1991).

NMDA receptors play a specialized role due to the unique properties of their linked ion channel and participate in various plastic neuronal events including the initiation of long-term potentiation (LTP; Collingridge et al. J. Physiol [Lond.] 334, 33, 1983), which is a proposed substrate of learning and memory (Morris et al. Nature, 319, 774, 1986) and the establishment of synaptic contacts during neuronal development (Kleinschmidt et al. Science 238, 355, 1987). NMDA receptors are also involved in other processes such as the transmission of sensory information (MacDermott and Dale, Trends Neurosci. 10, 280, 1987).

Apart from their important physiological roles, excitatory acidic amino acids are also involved in pathophysiological events in the CNS. Abnormally low levels of Glu can compromise normal level of excitation and cause, for instance, learning and memory deficits whereas excessive levels can produce toxic effects. The term "excitotoxicity" was coined by Olney (in Hyhan W.L. [ed]: "Heritage Disorders of Amino Acids Metabolism" New York: Macmillar pp. 501-512, 1989) to describe the process by which excitatory amino acids can cause neuronal cell death. This process is mainly

mediated by the stimulation of NMDA receptors and may occur in cases of cerebral ischaemia, stroke, hypoglycaemia, epilepsy, Alzheimer's disease, Huntington's disease, anxiety and schizophrenia.

The mechanism believed to be involved is represented in schematic form in Figure 1 (modified from Faoaqui and Horrocks, Brain Res. Rev. 16, 171-191, 1991). The NMDA receptor comprises several binding domains that interact between each other for a proper functioning and modulation of nerve cell activity. As shown in Figure 1, it is theorized that the NMDA receptor forms a complex acting as a receptor-linked ion channel. Essentially, the function of the receptor is to bind NMDA or the natural amino acid, Glu, and open an associated ion channel that allows the entry of Na^+, K^+ and Ca^{++} into the stimulated neuron. Whereas the ion channels of other excitatory amino acid receptors (AMPA, kainate, L-AP4 and metabotropic) are only permeable to Na^+ and K^+, the NMDA receptor channel is also permeable to Ca^{++}. This feature is of some importance for the proposed role of this receptor in both short and long-term plasticity (learning and memory) and neuropathology. A second unique feature of the NMDA receptor ion channel is a voltage dependent block by Mg^{++} (Nowak, Nature 307, 462, 1984). Upon low level of CNS activity, the NMDA receptor is most likely silent due to the physiological concentration of extracellular Mg^{++} which blocks the NMDA-linked ion channel. CNS activation may first be mediated by the stimulation of other Glu receptors such as the AMPA receptor, causing post-synaptic cell depolarization. Upon depolarization, the driving force for Mg^{++} to enter the NMDA channel is reduced and the block is relieved. This property is particularly important for processes involved in LTP and developmental plasticity. The NMDA receptor can also be blocked by organic molecules such as the dissociative anaesthetic, phencyclidine (PCP). Dissociative anaesthetics do not compete with agonists (such as Glu) at the transmitter recognition site of the NMDA receptor, but are un-competitive antagonists. Their blocking effect requires agonist activa-

tion of the NMDA receptor and thus it is recognized to be "use dependent". Therefore, it is believed that the PCP binding domain resides inside the lumen of the NMDA ion channel.

The conformation of the NMDA receptor can also be allosterically modified by the stimulation of a strychnine-insensitive glycine (Gly) binding domain. Gly markedly facilitates NMDA receptor responses. Several substances have effects at the NMDA receptor which cannot be explained by an interaction with the sites described above. These include spermidine, Zn^{++} and tricyclic antidepressants. Spermidine facilitates the stimulation by Glu (or NMDA; Fig. 1) while Zn^{++} and tricyclic antidepressants are potent blockers of the NMDA receptor (not shown).

Regulation of intracellular Ca^{++}-dependent activity by NMDA is also shown in Figure 1. Secondary responses mediated via a rise in intracellular Ca^{++} concentration include the stimulation of phospholipase C (PLC) to produce diacylglycerol (DAG) and subsequent protein kinase C (PKC) activation as well as the production of arachidonic acid (AA) and eicosanoids. Phosphorylation of membrane proteins by PKC may play a role in the opening of the ion channel. It is also known that an overstimulation of brain NMDA receptors can be caused by anoxia, ischaemia and hypoglycaemia, resulting in a build-up of the concentration of Ca^{++} in the stimulated neuron and a cascade of intracellular events (activation of phospholipases [PLA_2, PLC], lipases, proteases and endonucleases; Fig 1) that cause neuronal cell death (Farooqui and Horrocks, Brain Res. Rev. 16, 171; 1991).

The various domains of the NMDA receptor complex can be regarded as potential sites for pharmacological manipulation of receptor function. Specific antagonists of the NMDA and Gly binding domains and ligands of the PCP receptor have been proposed to protect against an overstimulation of the NMDA receptor. In this respect, PCP is considered as a good neuroprotecting agent and a potent anticonvulsant (Rogawski and Porter, Pharm. Rev. 42, 223; 1990). Unfortunately, PCP also binds to other CNS receptors (not linked to the NMDA

receptor complex), causing profound psychotic effects manifested by hallucinations, depersonalization, maniacal excitement and delirium.

On the other hand, Khangari et. al. (Science 225,76-78,1984 and in "Sigma and PCP-like compounds as molecular probes in biology", eds. E.F. Domino and J.-M. Kamenka, NPP Books, Ann Arbor pp. 673-685, 1988) have indicated that cells of the lymphoid system (lymphocytes, monocytes, macrophages) possess specific receptors for PCP which when stimulated cause suppression of DNA synthesis and decrease in cytokine and immunoglobulin production. Compounds that block or mimic the effect of PCP on lymphoid cells may be of some beneficial value for the regulation of immune host-defense mechanisms.

There is therefore a need for other compounds which, while binding to the NMDA receptor complex or otherwise, will protect neurons against NMDA receptor-induced degeneration and/or enhance host-defence immune responses. It is hoped that the development of such compounds will lead to the design of effective neuroprotective and/or immunostimulatory agents, devoid of undesired side-effects. There is also a need for other compounds whose action will be to enhance the activity of NMDA receptor stimulation. These latter compounds may find practical uses for the treatment of neuropathological disorders related to learning and memory processes.

OBJECTS OF THE INVENTION

An object of the invention is to provide a natural peptide displaying NMDA receptor antagonist and immunostimulatory activities.

Another object of the invention is to provide a series of synthetic peptides with potent antagonist activity against NMDA receptor stimulation.

Another object of the invention is to provide a series of synthetic peptides capable of acting as potentiators of NMDA receptor stimulation.

Another object of the invention is to provide a series of synthetic peptides with immunostimulatory properties.

Yet another object of the invention is to use natural and

synthetic peptides for treating or preventing CNS and/or immune diseases in mammals.

SUMMARY OF THE INVENTION

The present invention relates to a natural peptide consisting of 15 amino acids which can be isolated from the adrenal medulla of mammals and has been designated "histogranin-(1-15)" [SEQ ID NO:1] or, more simply, "histogranin" [SEQ ID NO:1], and to synthetic analogs of histogranin [SEQ ID NO:1] exhibiting NMDA receptor antagonist or agonist effects and/or immunostimulatory activities, and to their experimental and medicinal uses for the regulation of neuronal or immune functions.

More particularly, according to one aspect of the invention there is provided a peptide designated "histogranin-(1-15)" or "histogranin" [SEQ ID NO:1] having the structure: H-Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH, in substantially pure form.

According to another aspect of the invention, there is provided a series of synthetic peptides having a structure according to the following general formula: H-R_1-Gln-Gly-Arg-R_2-CO-R_3 wherein: R_1 represents one structure selected from the group consisting of: X-Asn-Tyr-Ala-Leu-Lys-Gly, X being an hydroxyl-containing amino acid; Y-Asn-Tyr-Ala-Leu-Lys-Gly, Y being a hydrocarbon side chain-containing amino acid; Z-Asn-Tyr-Ala-Leu-Lys-Gly, Z being an aromatic amino acid; W-Asn-Tyr-Ala-Leu-Lys-Gly, W being a sulfur-containing amino acid; A-Asn-Tyr-Ala-Leu-Lys-Gly; Ser-A-Tyr-Ala-Leu-Lys-Gly; Ser-Asn-Tyr-Ala-Leu-Lys-A; A-Tyr-Ala-Leu-Lys-Gly; Asn-Tyr-Ala-Leu-Lys-A; Tyr-Ala-Leu-A-Gly; Ala-Leu-A-Gly; Leu-A-Gly; A-Gly; and Val-Val-Tyr-Ala-Leu-Lys-A-, A being a basic amino acid: R_2 represents one structure selected from the group consisting of: a single covalent bond (no intervening amino acids); Thr-Leu; Thr-Leu-Tyr-Gly-Phe; Thr-Leu-Tyr-Gly-Phe-Cys and Thr-Leu-Tyr-Gly-Phe-Gly-Gly; and R_3 represents a radical selected from the group consisting of -OH and $-NH_2$.

The invention also relates to processes for extracting and synthesizing such compounds and analogs.

The peptides of the invention are capable of exhibiting phencyclidine (PCP)-like agonist or antagonist activities blocking or potentiating brain N-methyl-D-aspartate (NMDA) receptor stimulation and enhancing peripheral host-defense immune responses. This makes the peptides suitable for the management of neurodegenerative and/or immune diseases.

DETAILED DESCRIPTION OF THE INVENTION

I. STRUCTURE OF HISTOGRANIN AND ANALOGS THEREOF

The natural peptide histogranin-(1-15) [SEQ ID NO:1] has the structure:

H-Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH.

The natural peptide, referred to hereinafter simply as "histogranin," [SEQ ID NO:1] may be extracted from a natural source, e.g. bovine adrenal medulla, or synthesized using the solid-phase procedure for peptide synthesis. The peptide is mostly concentrated in the pituitary and the adrenal medulla. The synthetic peptide displays the same analytical criteria as the natural compound and it antagonizes the action of NMDA in the brain.

The interaction of histogranin [SEQ ID NO:1] with the NMDA receptor complex is shown in vitro by its allosteric interaction with the PCP binding domain and in vivo by its blockade of NMDA-induced convulsion. Histogranin [SEQ ID NO:1] binds to a site of rat brain membrane preparations that fulfills the criteria of a neuropeptide receptor namely, high affinity, specificity, saturability and reversibility. In rat brain, the distribution profile of the histogranin receptor parallels that of the selective PCP receptor ligand, [^{3}H]MK-801, a potent non-competitive antagonist of the NMDA receptor. There is a higher concentration of both PCP and histogranin receptors in hippocampus and cortex while cerebellum and striatum display lower binding activities. Additional evidence supporting an interaction of histogranin with the

NMDA receptor complex arises from the observation that histogranin potentiates the binding of [^{3}H]dextromethorphan, a drug known to block NMDA-operated ion channels. Moreover, the potentiation of [^{3}H]dextromethorphan binding by histogranin is blocked by a saturating concentration of NMDA.

In vivo, histogranin [SEQ ID NO:1] (1-6 nmol, i.c.v. in mice) is a potent blocker of NMDA- and AMPA-induced convulsions and these effects are selective, not being observed with kainate, bicuculline and pentylene tetrazole induced convulsions. The blockade of AMPA-induced convulsion by histogranin may result from its direct interaction with the AMPA receptor or, indirectly, from a blockade of the NMDA receptor-mediated component of the AMPA response. AMPA may act as a primer of the NMDA response, relieving the voltage-dependent Mg^{++} block and thus, allowing the stimulation of the NMDA receptor by endogenous Glu. Receptor binding studies indicate that histogranin interacts with the NMDA receptor complex but not with the AMPA receptor, suggesting that its blockade of AMPA-induced convulsion is indirect.

In periphery, histogranin [SEQ ID NO:1] is mainly concentrated in the adrenal medulla and in organs (spleen, lung) where important immune responses in host-defense mechanisms take place. The peripheral localization of histogranin is in accordance with its marked potentiation of interleukin-1α (IL-1α) mRNA production and IL-1 secretion by alveolar macrophages in response to lipopolysaccharide (LPS).

Various synthetic fragments and analogs of histogranin have been tested for their ability to bind to the brain histogranin receptor and to inhibit the electrically-induced contractions of the guinea pig ileum (GPI), a rapid peripheral screening test for measuring the bioactivity of peptide hormones. The relevance of this test is supported by its sensitivity to [Ser^1]histogranin [SEQ ID NO:2], a potent blocker of NMDA-induced convulsion in mice. Moreover, the GPI also contains the NMDA receptor (Shannon and Sawyer J. Pharmacol. Exp. Ther. 251, 518-523; 1989). The results indicate that histogranin agonists (or NMDA receptor blockers)

can be designed based on the following structures:

a) [X^1]histogranin wherein X is a hydroxyl(-OH)-containing amino acid (e.g. Ser and Thr).

b) [Y^1]histogranin wherein Y stands for a hydrocarbon side-chain-containing amino acid (e.g. Gly, Leu, Ala, Val, Ile).

c) [Z^1]histogranin wherein Z is an aromatic amino acid (e.g. Phe, Tyr).

d) [W^1]histogranin wherein W is a sulfur(-SH)-containing amino acid (e.g. Met, Cys).

e) [X^1]histogranin-(1-12) and [X^1]histogranin-(1-10) wherein X is defined as in (a).

f) [Y^1]histogranin-(1-12) and [Y^1]histogranin-(1-10) wherein Y is defined as in (b).

g) [Z^1]histogranin-(1-12) and [Z^1]histogranin-(1-10) wherein Z is defined as in (c).

h) [W^1]histogranin-(1-12) and [W^1] histogranin-(1-10) wherein W is defined as in (d).

i) C-terminal amidated histogranins and histogranin fragments as defined under (a), (b), (c), (d), (e), (f), (g) and (h).

The results also indicate that histogranin antagonists (or NMDA receptor potentiating agents) can be designed based on the following structures:

a) [A^1]histogranin wherein A stands for a basic amino acid.

b) [A^1]histogranin-(1-12) and [A^1]histogranin-(1-10) wherein A is defined as in (a).

c) [Ser^1, A^2]histogranin wherein A is defined as in (a).

d) [Ser^1, A^2]histogranin-(1-12) and [Ser^1, A^2]histogranin(1-10) wherein A is defined as in (a).

e) [Ser^1, A^7]histogranin wherein A is defined as in (a).

f) [Ser^1, A^7]histogranin-(1-12) and [Ser^1, A^7]histogranin-(1-10) wherein A is defined as in (a).

g) [A^2]histogranin-(2-15) wherein A is defined as in (a).

h) [A^2]histogranin-(2-12) and [A^2]histogranin-(2-10) wherein A is defined as in (a).

i) [A^7]histogranin-(2-15) wherein A is defined as in (a).

j) [A^7]histogranin-(2-12) and [A^7]histogranin-(2-10) wherein A is defined as in (a).

k) [A^6]histogranin-(3-15) wherein A is defined as in (a).

l) [A^6]histogranin-(3-12) and [A^6]histogranin-(3-10) wherein A is defined as in (a).

m) [A^6]histogranin-(4-15) wherein A is defined as in (a).

n) [A^6]histogranin-(4-12) and [A^6]histogranin-(4-10) wherein A is defined as in (a).

o) [A^6]histogranin (5-15) wherein A is defined as in (a).

p) [A^6]histogranin-(5-12) and [A^6]histogranin-(5-10) wherein A is defined as in (a)

q) [A^6]histogranin-(6-15) wherein A is defined as in (a).

r) [A^6]histogranin-(6-12) and [A^6]histogranin-(6-10) wherein A is defined as in (a).

s) [Val^1, Val^2, A^7]-histogranin wherein A is defined as in (a).

t) [X^1]-, [Y^1]-, [Z^1]- and [W^1]-histogranin-Gly^{16}-Gly^{17} wherein X, Y, Z and W are defined as in (a), (b), (c) and (d) of the previous section, respectively.

u) [X^1]-, [Y^1]-, [Z^1]- and [W^1]-histogranin-Cys^{16} wherein X, Y, Z and W are defined as in (a), (b), (c) and (d) of the previous section, respectively.

x) C-terminal amidated histogranin analogs and fragments as defined under (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n), (o), (p), (q), (r), (s), (t) and (u).

The above listed peptides are proposed on the basis that some modifications in the structure of histogranin like the introduction of a hydroxyl-containing amino acid (Ser or Thr) in position 1 give rise to active compounds in both the GPI and rat brain binding assays while other changes like the introduction of a basic amino acid (Arg or Lys) in position 1 destroy the bioactivity on the GPI assay but not the receptor binding activity. The compounds of the first class are considered as putative agonists while those of the second class are potential antagonists (see below for biological activity). On the other hand, the [Ser^1]histogranin fragments

(1-10) [SEQ ID NO:23] and (1-12) [SEQ ID NO:12] are potent anticonvulsants in the mouse model.

The following specific peptides are believed to be useful agonists or antagonists, although some of the compounds listed as agonists may be more effective as antagonists, and vice versa.

Agonists

HN	[SEQ ID NO:1]
HN-amide	[SEQ ID NO:1]
[Ser^1]HN	[SEQ ID NO:2]
[Ser^1]HN-amide	[SEQ ID NO:2]
[Thr^1]HN	[SEQ ID NO:3]
[Thr^1]HN-amide	[SEQ ID NO:3]
[Gly^1]HN	[SEQ ID NO:4]
[Gly^1]HN-amide	[SEQ ID NO:4]
[Leu^1]HN	[SEQ ID NO:5]
[Leu^1]HN-amide	[SEQ ID NO:5]
[Ala^1]HN	[SEQ ID NO:6]
[Ala^1]HN-amide	[SEQ ID NO:6]
[Val^1]HN	[SEQ ID NO:7]
[Val^1]HN-amide	[SEQ ID NO:7]
[Ile^1]HN	[SEQ ID NO:8]
[Ile^1]HN-amide	[SEQ ID NO:8]
[Phe^1]HN	[SEQ ID NO:9]
[Phe^1]HN-amide	[SEQ ID NO:9]
[Tyr^1]HN	[SEQ ID NO:10]
[Tyr^1]HN-amide	[SEQ ID NO:10]
[Cys^1]HN	[SEQ ID NO:11]
[Cys^1]HN-amide	[SEQ ID NO:11]
[Ser^1]HN-(1-12)	[SEQ ID NO:12]
[Ser^1]HN-(1-12)-amide	[SEQ ID NO:12]
[Thr^1]HN-(1-12)	[SEQ ID NO:13]
[Thr^1]HN-(1-12)-amide	[SEQ ID NO:13]
[Gly^1]HN-(1-12)	[SEQ ID NO:14]
[Gly^1]HN-(1-12)-amide	[SEQ ID NO:14]
[Leu^1]HN-(1-12)	[SEQ ID NO:15]

[Leu^1]HN-(1-12)-amide	[SEQ ID NO:15]
[Ala^1]HN-(1-12)	[SEQ ID NO:16]
[Ala^1]HN-(1-12)-amide	[SEQ ID NO:16]
[Val^1]HN-(1-12)	[SEQ ID NO:17]
[Val^1]HN-(1-12)-amide	[SEQ ID NO:17]
[Ile^1]HN-(1-12)	[SEQ ID NO:18]
[Ile^1]HN-(1-12)-amide	[SEQ ID NO:18]
[Phe^1]HN-(1-12)	[SEQ ID NO:19]
[Phe^1]HN-(1-12)-amide	[SEQ ID NO:19]
[Tyr^1]HN-(1-12)	[SEQ ID NO:20]
[Tyr^1]HN-(1-12)-amide	[SEQ ID NO:20]
HN-(1-12)	[SEQ ID NO:21]
HN-(1-12)-amide	[SEQ ID NO:21]
[Cys^1]HN-(1-12)	[SEQ ID NO:22]
[Cys^1]HN-(1-12)-amide	[SEQ ID NO:22]
[Ser^1]HN-(1-10)	[SEQ ID NO:23]
[Ser^1]HN-(1-10)-amide	[SEQ ID NO:23]
[Thr^1]HN-(1-10)	[SEQ ID NO:24]
[Thr^1]HN-(1-10)-amide	[SEQ ID NO:24]
[Gly^1]HN-(1-10)	[SEQ ID NO:25]
[Gly^1]HN-(1-10)-amide	[SEQ ID NO:25]
[Leu^1]HN-(1-10)	[SEQ ID NO:26]
[Leu^1]HN-(1-10)-amide	[SEQ ID NO:26]
[Ala^1]HN-(1-10)	[SEQ ID NO:27]
[Ala^1]HN-(1-10)-amide	[SEQ ID NO:27]
[Val^1]HN-(1-10)	[SEQ ID NO:28]
[Val^1]HN-(1-10)-amide	[SEQ ID NO:28]
[Ile^1]HN-(1-10)	[SEQ ID NO:29]
[Ile^1]HN-(1-10)-amide	[SEQ ID NO:29]
[Phe^1]HN-(1-10)	[SEQ ID NO:30]
[Phe^1]HN-(1-10)-amide	[SEQ ID NO:30]
[Tyr^1]HN-(1-10)	[SEQ ID NO:31]
[Tyr^1]HN-(1-10)-amide	[SEQ ID NO:31]
HN-(1-10)	[SEQ ID NO:32]
HN-(1-10)-amide	[SEQ ID NO:32]
[Cys^1]HN-(1-10)	[SEQ ID NO:33]
[Cys^1]HN-(1-10)-amide	[SEQ ID NO:33]

Antagonists

[Arg^1]HN	[SEQ ID NO:34]
[Arg^1]HN-amide	[SEQ ID NO:34]
[Arg^1]HN-(1-12)	[SEQ ID NO:35]
[Arg^1]HN-(1-12)-amide	[SEQ ID NO:35]
[Arg^1]HN-(1-10)	[SEQ ID NO:36]
[Arg^1]HN-(1-10)-amide	[SEQ ID NO:36]
[Ser^1, Arg^2]HN	[SEQ ID NO:37]
[Ser^1, Arg^2]HN-amide	[SEQ ID NO:37]
[Ser^1, Arg^2]HN-(1-12)	[SEQ ID NO:38]
[Ser^1, Arg^2]HN-(1-12)-amide	[SEQ ID NO:38]
[Ser^1, Arg^2]HN-(1-10)	[SEQ ID NO:39]
[Ser^1, Arg^2]HN-(1-10)-amide	[SEQ ID NO:39]
[Ser^1, Arg^7]HN	[SEQ ID NO:40]
[Ser^1, Arg^7]HN-amide	[SEQ ID NO:40]
[Ser^1, Arg^7]HN-(1-12)	[SEQ ID NO:41]
[Ser^1, Arg^7]HN-(1-12)-amide	[SEQ ID NO:41]
[Ser^1, Arg^7]HN-(1-10)	[SEQ ID NO:42]
[Ser^1, Arg^7]HN-(1-10)-amide	[SEQ ID NO:42]
[Arg^2]HN-(2-15)	[SEQ ID NO:43]
[Arg^2]HN-(2-15)-amide	[SEQ ID NO:43]
[Arg^2]HN-(2-12)	[SEQ ID NO:44]
[Arg^2]HN-(2-12)-amide	[SEQ ID NO:44]
[Arg^2]HN-(2-10)	[SEQ ID NO:45]
[Arg^2]HN-(2-10)-amide	[SEQ ID NO:45]
[Arg^7]HN-(2-15)	[SEQ ID NO:46]
[Arg^7]HN-(2-15)-amide	[SEQ ID NO:46]
[Arg^7]HN-(2-12)	[SEQ ID NO:47]
[Arg^7]HN-(2-12)-amide	[SEQ ID NO:47]
[Arg^7]HN-(2-10)	[SEQ ID NO:48]
[Arg^7]HN-(2-10)-amide	[SEQ ID NO:48]
[Arg^6]HN-(3-15)	[SEQ ID NO:49]
[Arg^6]HN-(3-15)-amide	[SEQ ID NO:49]
[Arg^6]HN-(3-12)	[SEQ ID NO:50]
[Arg^6]HN-(3-12)-amide	[SEQ ID NO:50]
[Arg^6]HN-(3-10)	[SEQ ID NO:51]
[Arg^6]HN-(3-10)-amide	[SEQ ID NO:51]

[Arg^{6}]HN-(4-15)	[SEQ ID NO:52]
[Arg^{6}]HN-(4-15)-amide	[SEQ ID NO:52]
[Arg^{6}]HN-(4-12)	[SEQ ID NO:53]
[Arg^{6}]HN-(4-12)-amide	[SEQ ID NO:53]
[Arg^{6}]HN-(4-10)	[SEQ ID NO:54]
[Arg^{6}]HN-(4-10)-amide	[SEQ ID NO:54]
[Arg^{6}]HN-(5-15)	[SEQ ID NO:55]
[Arg^{6}]HN-(5-15)-amide	[SEQ ID NO:55]
[Arg^{6}]HN-(5-12)	[SEQ ID NO:56]
[Arg^{6}]HN-(5-12)-amide	[SEQ ID NO:56]
[Arg^{6}]HN-(5-10)	[SEQ ID NO:57]
[Arg^{6}]HN-(5-10)-amide	[SEQ ID NO:57]
[Arg^{6}]HN-(6-15)	[SEQ ID NO:58]
[Arg^{6}]HN-(6-15)-amide	[SEQ ID NO:58]
[Arg^{6}]HN-(6-12)	[SEQ ID NO:59]
[Arg^{6}]HN-(6-12)-amide	[SEQ ID NO:59]
[Arg^{6}]HN-(6-10)	[SEQ ID NO:60]
[Arg^{6}]HN-(6-10)-amide	[SEQ ID NO:60]
[Ser^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:61]
[Ser^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:61]
[Thr^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:62]
[Thr^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:62]
[Gly^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:63]
[Gly^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:63]
[Leu^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:64]
[Leu^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:64]
[Ala^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:65]
[Ala^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:65]
[Val^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:66]
[Val^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:66]
[Ile^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:67]
[Ile^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:67]
[Phe^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:68]
[Phe^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:68]
[Tyr^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:69]
[Tyr^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:69]
HN-Gly^{16}-Gly^{17}	[SEQ ID NO:70]

HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:70]
[Cys^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:71]
[Cys^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:71]
[Val^{1}, Val^{2}, Arg^{7}]HN	[SEQ ID NO:72]
[Val^{1}, Val^{2}, Arg^{7}]HN-amide	[SEQ ID NO:72]
[Lys^{1}]HN	[SEQ ID NO:73]
[Lys^{1}]HN-amide	[SEQ ID NO:73]
[Lys^{1}]HN-(1-12)	[SEQ ID NO:74]
[Lys^{1}]HN-(1-12)-amide	[SEQ ID NO:74]
[Lys^{1}]HN-(1-10)	[SEQ ID NO:75]
[Lys^{1}]HN-(1-10)-amide	[SEQ ID NO:75]
[Ser^{1}, Lys^{2}]HN	[SEQ ID NO:76]
[Ser^{1}, Lys^{2}]HN-amide	[SEQ ID NO:76]
[Ser^{1}, Lys^{2}]HN-(1-12)	[SEQ ID NO:77]
[Ser^{1}, Lys^{2}]HN-(1-12)-amide	[SEQ ID NO:77]
[Ser^{1}, Lys^{2}]HN-(1-10)	[SEQ ID NO:78]
[Ser^{1}, Lys^{2}]HN-(1-10)-amide	[SEQ ID NO:78]
[Ser^{1}, Lys^{7}]HN	[SEQ ID NO:79]
[Ser^{1}, Lys^{7}]HN-amide	[SEQ ID NO:79]
[Ser^{1}, Lys^{7}]HN-(1-12)	[SEQ ID NO:80]
[Ser^{1}, Lys^{7}]HN-(1-12)-amide	[SEQ ID NO:80]
[Ser^{1}, Lys^{7}]HN-(1-10)	[SEQ ID NO:81]
[Ser^{1}, Lys^{7}]HN-(1-10)-amide	[SEQ ID NO:81]
[Lys^{2}]HN-(2-15)	[SEQ ID NO:82]
[Lys^{2}]HN-(2-15)-amide	[SEQ ID NO:82]
[Lys^{2}]HN-(2-12)	[SEQ ID NO:83]
[Lys^{2}]HN-(2-12)-amide	[SEQ ID NO:83]
[Lys^{2}]HN-(2-10)	[SEQ ID NO:84]
[Lys^{2}]HN-(2-10)-amide	[SEQ ID NO:84]
[Lys^{7}]HN-(2-15)	[SEQ ID NO:85]
[Lys^{7}]HN-(2-15)-amide	[SEQ ID NO:85]
[Lys^{7}]HN-(2-12)	[SEQ ID NO:86]
[Lys^{7}]HN-(2-12)-amide	[SEQ ID NO:86]
[Lys^{7}]HN-(2-10)	[SEQ ID NO:87]
[Lys^{7}]HN-(2-10)-amide	[SEQ ID NO:87]
HN-(3-15)	[SEQ ID NO:88]
HN-(3-15)-amide	[SEQ ID NO:88]

HN-(3-12)	[SEQ ID NO:89]
HN-(3-12)-amide	[SEQ ID NO:89]
HN-(3-10)	[SEQ ID NO:90]
HN-(3-10)-amide	[SEQ ID NO:90]
HN-(4-15)	[SEQ ID NO:91]
HN-(4-15)-amide	[SEQ ID NO:91]
HN-(4-12)	[SEQ ID NO:92]
HN-(4-12)-amide	[SEQ ID NO:92]
HN-(4-10)	[SEQ ID NO:93]
HN-(4-10)-amide	[SEQ ID NO:93]
HN-(5-15)	[SEQ ID NO:94]
HN-(5-15)-amide	[SEQ ID NO:94]
HN-(5-12)	[SEQ ID NO:95]
HN-(5-12)-amide	[SEQ ID NO:95]
HN-(5-10)	[SEQ ID NO:96]
HN-(5-10)-amide	[SEQ ID NO:96]
HN-(6-15)	[SEQ ID NO:97]
HN-(6-15)-amide	[SEQ ID NO:97]
HN-(6-12)	[SEQ ID NO:98]
HN-(6-12)-amide	[SEQ ID NO:98]
HN-(6-10)	[SEQ ID NO:99]
HN-(6-10)-amide	[SEQ ID NO:99]
[Ser^{1}]HN-Cys^{16}	[SEQ ID NO:100]
[Ser^{1}]HN-Cys^{16}-amide	[SEQ ID NO:100]
[Thr^{1}]HN-Cys^{16}	[SEQ ID NO:101]
[Thr^{1}]HN-Cys^{16}-amide	[SEQ ID NO:101]
[Gly^{1}]HN-Cys^{16}	[SEQ ID NO:102]
[Gly^{1}]HN-Cys^{16}-amide	[SEQ ID NO:102]
[Leu^{1}]HN-Cys^{16}	[SEQ ID NO:103]
[Leu^{1}]HN-Cys^{16}-amide	[SEQ ID NO:103]
[Ala^{1}]HN-Cys^{16}	[SEQ ID NO:104]
[Ala^{1}]HN-Cys^{16}-amide	[SEQ ID NO:104]
[Val^{1}]HN-Cys^{16}	[SEQ ID NO:105]
[Val^{1}]HN-Cys^{16}-amide	[SEQ ID NO:105]
[Ile^{1}]HN-Cys^{16}	[SEQ ID NO:106]
[Ile^{1}]HN-Cys^{16}-amide	[SEQ ID NO:106]
[Phe^{1}]HN-Cys^{16}	[SEQ ID NO:107]

[Phe^1]HN-Cys^{16}-amide	[SEQ ID NO:107]
[Tyr^1]HN-Cys^{16}	[SEQ ID NO:108]
[Tyr^1]HN-Cys^{16}-amide	[SEQ ID NO:108]
HN-Cys^{16}	[SEQ ID NO:109]
HN-Cys^{16}-amide	[SEQ ID NO:109]
[Cys^1]HN-Cys^{16}	[SEQ ID NO:110]
[Cys^1]HN-Cys^{16}-amide	[SEQ ID NO:110]
[Val^1, Val^2, Lys^7]HN	[SEQ ID NO:111]
[Val^1, Val^2, Lys^7]HN-amide	[SEQ ID NO:111]

Other useful peptides

HN-(2-15)	[SEQ ID NO: 112]
HN-(2-15)-amide	[SEQ ID NO: 112]
HN-(7-15)	[SEQ ID NO: 113]
HN-(7-15)-amide	[SEQ ID NO: 113]
HN-(8-15)	[SEQ ID NO: 114]
HN-(8-15)-amide	[SEQ ID NO: 114]
[Ser^1]HN-(1-14)	[SEQ ID NO: 115]
[Ser^1]HN-(1-14)-amide	[SEQ ID NO: 115]
[Ser^1]HN-(1-13)	[SEQ ID NO: 116]
[Ser^1]HN-(1-13)-amide	[SEQ ID NO: 116]
[Ser^1, His^2]HN	[SEQ ID NO: 117]
[Ser^1, His^2]HN-amide	[SEQ ID NO: 117]
[Gln^1]HN	[SEQ ID NO: 118]
[Gln^1]HN-amide	[SEQ ID NO: 118]
[Ser^1, Ala^2]HN	[SEQ ID NO: 119]
[Ser^1, Ala^2]HN-amide	[SEQ ID NO: 119]
[Ser^1, Ser^2]HN	[SEQ ID NO: 120]
[Ser^1, Ser^2]HN-amide	[SEQ ID NO: 120]
[Glu^1]HN	[SEQ ID NO: 121]
[Glu^1]HN-amide	[SEQ ID NO: 121]
[Ser^1]HN-Gly^{16}	[SEQ ID NO: 122]
[Ser^1]HN-Gly^{16}-amide	[SEQ ID NO: 122]
[Ser^1, His^2]HN	[SEQ ID NO: 123]
[Ser^1, His^2]HN-amide	[SEQ ID NO: 123]
HN-(2-10)	[SEQ ID NO: 124]
HN-(2-10)-amide	[SEQ ID NO: 124]

[Arg^2]HN	[SEQ ID NO: 125]
[Arg^2]HN-amide	[SEQ ID NO: 125]
[Lys^2]HN	[SEQ ID NO: 126]
[Lys^2]HN-amide	[SEQ ID NO: 126]
[$pGlu^1$]HN	[SEQ ID NO: 127]
[$pGlu^1$]HN-amide	[SEQ ID NO: 127]

II. USES OF HISTOGRANIN AND ANALOGS

Histogranin agonists and antagonists may find beneficial therapeutical use in various CNS disorders that imply either an overstimulation or a substimulation of brain NMDA receptors. These include: learning and memory deficits, cerebral stroke, cerebral ischaemia, epilepsy, Alzheimer's disease, Huntington's disease, traumatic brain injury and other neurodegenerative disorders. The link between the modulation of NMDA receptor activity and the treatment of these diseases has been fully documented (Olney, Ann. Rev. Pharmacol. Toxicol., 30: 47-71, 1990; Foster et al., in "Current and future Trends in Anticonvulsant, Anxiety and Stroke Therapy" Wiley-Liss Inc., 301-329, 1990; Bogawski and Porter, Pharmacol. Rev., 42: 223-286, 1990). The above listed compounds may also find some use in modulating the phenomena of opioid tolerance and dependence (Trujillo and Akil, Science, 251: 85-87, 1991; Marek et al., Brain Res., 547: 77-81, 1991; Marek et al., Brain Res., 558: 163-165, 1991; Ben-Eliyahu et al., Brain Res., 575: 304-308, 1992; Tiseo and Inturrisi, J. Pharmacol. Exp. Ther., 264: 1090-1096, 1993). According to the above mentioned studies, NMDA receptor antagonists not only prevent tolerance and dependence but they also alleviate these phenomena once established. The above listed compounds may also find use in modulating learning and memory processes as well as immune functions.

III. EXTRACTION OF HISTOGRANIN

As indicated above, histogranin [SEQ ID NO:1] is a peptide of 15 amino acids which has been shown to be concentrated mainly in the mammalian pituitary and adrenal

medulla, but high levels have been detected in peripheral organs (spleen, lung) where host-defense immune reactions are known to take place. Brain and blood plasma contain low but significant levels of the peptide and there appear to be high concentrations in particular areas of the brain. As far as concentration in subcellular fractions (nucleus, granules, mitochondria, microsomes, cytosol, etc.) is concerned, the peptide is more concentrated in the secretory granules than in other fractions.

Chemical stimulation of the perfused bovine adrenal gland, e.g. with carbamylcholine (an artificial stress condition), evokes large releases of the immunoreactive histogranin [SEQ ID NO:1] (110.5 ± 12 pmol/30 min/gland) as detected by radioimmunoassay (RIA) in the perfusate after its isolation by high performance liquid chromatography (HPLC) on a μ-Bondapak C18 column. Upon stimulation with carbamylcholine, the release of histogranin is generally seen after the release of catecholamines and enkephalins (5 to 10 min after the release of catecholamines and/or enkephalins which are seen immediately after stimulation) and lasts for a longer period of time (up to 40 min after the beginning of the 1.5 min pulse stimulation as compared with 10 min for catecholamines).

Histogranin [SEQ ID NO:1] may be extracted from bovine adrenal medulla following a purification procedure similar to that described for the isolation of neuromedin C (Lemaire et. al., Peptides 10, 355; 1989) involving extraction with an acid solution, lyophilization of the extract and purification by partition chromatography and HPLC. Alternatively, because of the high concentration of the peptide in the secretory granules and the secretion therefrom, the peptide may be extracted from perfusate of carbamylcholine-stimulated bovine adrenal glands, e.g. with Krebs solution (pH 7.4), followed by isolation and purification by HPLC (SP-5PW and μ-Bondapak C18 columns, Waters) as described by Lemaire et. al. (Peptides 10, 355; 1989).

Amino acid analysis of the purified material after HCl

digestion indicates the following ratios:

Asp, 1.5 (1); Glu, 1.3 (1); Met, 0.8 (1); Gly, 2.8 (3); Thr, 1.0 (1); Ala, 0.7 (1); Arg, 1.0 (1); Tyr, 1.6 (2); Leu, 1.8 (2); Phe, 1.2 (1); and Lys, 0.8 (1).

Amino acid sequencing showed the structure of the peptide to be as indicated above and in accordance with the amino acid composition. The name "histogranin" was adopted for this peptide because of its high degree of homology (80%) with fragment-(86-100) of histone H4 (-Val-Val-Tyr-Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe- [SEQ ID NO:72]; the underlined amino acids being common to both peptides) and due to its high concentration in adrenal chromaffin granules.

IV. SYNTHESIS OF HISTOGRANIN AND ANALOGS

The natural peptide and novel analogs can be synthesized, e.g. by the solid-phase procedure as described by Lemaire et. al., Int. J. Peptide Protein Res., 27, 300; 1986 and Turcotte et. al. Int. J. Peptide Protein Res. 23, 361-367;1984, followed by cleavage from the solid support (resin) and purification by preparative or semi-preparative HPLC. The purity and identity of the synthetic peptides can be verified by thin layer chromatography, analytical HPLC and fast atom bombardment (FAB) mass spectrometry.

Synthetic histogranin [SEQ ID NO:1] possesses the same analytical criteria as the natural compound, as shown by its retention time in three distinct HPLC systems (μ-Bondapak, SP-5PW, Zorbax ODS) and its amino acid composition. Synthetic histogranin also cross-reacts with the anti-bombesin antibody that has been used for the detection and isolation of the natural peptide from bovine adrenal medulla (while most synthetic histogranin analogs do not cross-react with anti-bombesin).

A stable synthetic analog of histogranin ([Ser^1]histogranin [SEQ ID NO:2]) provides a good antigen for the development of a specific antibody for recognition of the natural peptide in bovine adrenal medulla and in various tissue extracts of rat (10 fmol limit sensitivity).

V. ACTIVITY OF HISTOGRANIN AND ANALOGS

Receptor binding activity. [^{125}I, Ser^{1}]histogranin [SEQ ID NO:2] binds to a high affinity saturable receptor in membrane preparations of rat brain. The binding of the labelled peptide is readily displaced by histogranin [SEQ ID NO:1], [Ser^{1}]histogranin [SEQ ID NO:2] and related peptides that retain the structural requirements of the receptor. [^{125}I, Ser^{1}]histogranin [SEQ ID NO:2] binding is not displaced by PCP, sigma, NMDA and AMPA receptor ligands, indicating that histogranin [SEQ ID NO:1] possesses its own receptor distinct from the NMDA, PCP, sigma and AMPA receptors. However, the biogenic amine, spermidine, does inhibit the binding of the labelled peptide, even though the binding of [^{3}H]spermidine itself is not inhibited by histogranin. These data indicate that the receptor of histogranin is similar but not identical to the spermidine binding domain present on the NMDA receptor complex (Fig. 1).

Histogranin [SEQ ID NO:1] (10^{-9} - 10^{-4} M) also potentiates the binding of the PCP/σ receptor ligand and NMDA receptor blocker, [^{3}H]dextromethorphan. Such modulation of [^{3}H]dextromethorphan binding to rat brain membranes is comparable to the effect of the anticonvulsant, phenytoin, on the binding of [^{3}H]dextromethorphan to membrane preparations of guinea pig brain (Musacchio et al., Mol. Pharmacol. 35, 1; 1989). The potentiations of the binding of [^{3}H]dextromethorphan by [Ser^{1}]histogranin [SEQ ID NO:2] and histogranin [SEQ ID NO:1] are biphasic, the first plateau (140-150%) being reached at concentrations between 10^{-9} and 10^{-8} M, the second potentiation being seen at concentrations exceeding 10^{-7} M. [Ser^{1}]histogranin [SEQ ID NO:2] appears to be slightly more potent than histogranin [SEQ ID NO:1] in this respect.

In order to determine what receptor site (PCP, σ and/or NMDA) is involved in the potentiation of [^{3}H]dextromethorphan binding by [Ser^{1}]histogranin [SEQ ID NO:2], the binding experiments have been performed in the presence of saturating concentrations of selective PCP (MK-801), σ [(+)pentazocine] or NMDA (CPP, NMDA) receptor ligands. The first phase of the

potentiation is selectively blocked by NMDA (100 μM), CPP (1 μM) and MK-801 (1 μM) but not by (+)pentazocine (1 μM), indicating that the NMDA receptor complex is involved in this action of [Ser^1]-histogranin [SEQ ID NO:2]. It therefore appears that, in vitro, histogranin [SEQ ID NO:1] and [Ser^1]histogranin [SEQ ID NO:2] are potent modulators of [^{3}H]dextromethorphan binding to the NMDA receptor complex.

Effect of histogranin on brain NMDA, PCP and sigma sites

Histogranin was tested for its possible modulatory effect on the binding of specific NMDA ([^{3}H]CGP 39653), PCP ([^{3}H]MK-801) and σ ([^{3}H](+)pentazocine) receptor ligands. A specific interaction of histogranin with the NMDA receptor was demonstrated by its potent inhibitory effect on [^{3}H]CGP 39653 binding. Addition of increasing concentrations of histogranin produced a shallow inhibition of the binding. Analysis of the Hill plot revealed the presence of two phases (Hill coefficient:0.3), a high affinity phase (IC_{50}:0.6 nM) representing 33% of the sites and a low affinity phase (IC_{50}:3,955 nM) representing 67% of the sites. High concentrations of histogranin (>10^{-6}M) also inhibited the binding of the PCP receptor ligand, [^{3}H]MK-801, but not that of the σ receptor ligand, [^{3}H](+)-pentazocine.

Anticonvulsive activity. The potentiation of the binding of [^{3}H]dextromethorphan, a compound known to have in vivo anticonvulsive activity, suggested that histogranin [SEQ ID NO:1] may also possess such activity. In the case of mice, intracerebro-ventricular administrations of low doses (10 and 50 nmol/mouse) of synthetic histogranin [SEQ ID NO:1] and [Ser^1]histogranin [SEQ ID NO:2] efficiently protect against NMDA-induced convulsions. The anticonvulsive activity resides between positions 1 and 10 of the peptide. The unrelated synthetic peptide, H-Ser-Leu-Ser-Lys-Leu-Gly-Asp-Val-Tyr-Cys-COOH is inactive at 100 nmole doses (not shown) while [Ser^1]histogranin-(1-12) [SEQ ID NO:12] and [Ser^1]histogranin-(1-10) [SEQ ID NO:23] are good protecting agents at 10 nmol

doses against NMDA receptor stimulation. [Ser^1]histogranin [SEQ ID NO:2] also blocks AMPA-induced convulsions in mice but it does not affect kainate, bicuculline and pentylene tetrazole induced convulsions. Histogranin [SEQ ID NO:1] may hypothetically be considered as an endogenous blocker of central NMDA receptor by its interaction on a specific binding domain linked to the NMDA receptor complex and thus constitute an efficient neuroprotective agent, released upon some stressful conditions from the pituitary, the adrenal medulla or some specific brain centres. The natural compound and related synthetic peptides may find useful therapeutical uses for the treatment of neurodegenerative diseases including epilepsy, Alzheimer's disease, stroke, Huntington's disease, CNS trauma and hypoxia.

The binding of histogranin [SEQ ID NO:1] to its own receptor more likely induces an allosteric change in the conformation of the PCP receptor localized inside the NMDA receptor ion channel. Such a change favours the binding of [^{3}H]dextromethorphan to this site, an effect that may be perceived <u>in vivo</u> by a blockade of NMDA receptor stimulation. The physiological role of histogranin [SEQ ID NO:1] is not known, but it may favour the binding of some unknown endogenous ligand(s) to the PCP receptor and block the passage of ions (Ca^{++}) through the NMDA receptor-linked ion channel. On the other hand, the allosteric change in the conformation of the PCP binding domain located in the lumen of the ion channel may <u>per se</u> produce an effective block of the channel.

The effect of histogranin [SEQ ID NO:1] on the NMDA receptor complex can be readily compared to that of PCP which is considered as a good protective agent against NMDA receptor stimulation (Rogawski and Porter, Pharmacol. Rev. <u>42</u>, 223;1990). However, it appears that histogranin [SEQ ID NO:1] may act more selectively: its receptor being unique and selective to histogranin and closely related peptides. Moreover, the distribution of the histogranin receptor in the brain parallels that of the selective marker ([^{3}H]MK-801) of the PCP binding domain on the NMDA receptor complex, both

receptors being concentrated in hippocampus and cortex.

Guinea pig ileum assay. The rat brain membrane binding activity of synthetic histogranin [SEQ ID NO:1] and analogs was compared with their ability to inhibit the electrically evoked contraction of the guinea pig ileum (GPI). The GPI assay provides a useful test to monitor the biological activity of the synthetic peptides. Some parallelism can be established between this test and the ability of the various peptides to bind to the brain receptor and to block NMDA-induced convulsion in the "in vivo" mouse model (at least for the peptides tested in this latter model).

In such tests, it is found that many of the histogranin analogs have similar activity to histogranin [SEQ ID NO:1] and thereof are potential agonists. It is found that the agonist activity is greatly dependent on the integrity of the C-terminal portion of the whole molecule. Addition of Cys^{16} or Gly^{16}-Gly^{17} destroy the activity of the peptide on the GPI assay but not in the rat brain receptor binding assay. These compounds are thus considered as potential antagonists. On the other hand, removal of the N-terminal amino acid (histogranin-(2-15) [SEQ ID NO:112]) or the C-terminal tripeptide ([Ser^1]histogranin-(1-12) [SEQ ID NO:12]) and pentapeptide ([Ser^1]histogranin-(1-10) [SEQ ID NO:23]) provide active compounds in both tests and good working models for developing biologically active histogranin fragments. Other histogranin receptor agonists can also be obtained by the replacement of the N-terminal amino acid by aromatic (Phe, Tyr), hydroxyl-containing (Ser, Thr), hydrocarbon side-chain containing (Gly, Ala, Leu) and sulfur-containing (Met) amino acids. Replacements of positions 1 or 2 by a basic amino acid (Arg) destroy the bioactivity of the compound in the GPI assay but not in the receptor binding test, providing putative histogranin receptor antagonists. The two activities reside within fragments (1-10) and (1-12), two potent anticonvulsants in the mouse model. These fragments are good working models for developing agonists and antagonists with reduced chain

length. Interestingly, histogranin-(6-10) [SEQ ID NO:23] had no intrinsic activity in the GPI assay but potently inhibited the binding of [^{125}I][Ser^1]histogranin, providing a putative antagonist with reduced chain length. Finally, blockade of the carboxyl group by amidation (e.g. [Ser^1]histogranin-amide [SEQ ID NO:2]) increases the potency of the peptide on the GPI (135% as compared with [Ser^1]histogranin [SEQ ID NO:2]). Accordingly, useful changes in the structure of histogranin are proposed (see above section I).

The minimal core of histogranin that is responsible for its binding activity is a pentapeptide (histogranin-(6-10) [SEQ ID NO:99]); the minimal core for its agonist activity appears to be fragment-(1-10) [SEQ ID NO:32]. Changes in the structure of these shortened peptides can be made to increase their biological activity and/or allow their free passage into the central nervous system (cyclization, introduction of hydrophobic side-chains, etc.). It is believed that the use of such peptides, whose structure is derived from that of a natural compound, may give rise to the production of novel anticonvulsants and/or neuroprotective agents, devoid of the known side-effects of existing drugs.

One of the major concerns in using blockers of the NMDA receptor as potent anticonvulsants or neuroprotective agents is the induction of potential learning and memory deficits as well as PCP-like psychotic effects (hallucinations, depersonalization etc.). In this respect, histogranin [SEQ ID NO:1] or related peptides may be more selective and their putative clinical use may be justified in potentially life-threatening situations (stroke etc.).

Peripheral immune activity. The particular high concentration of histogranin [SEQ ID NO:1] in some peripheral tissues (spleen, lung) that are known to be the sites of immune host-defense reactions suggests a role for this peptide in the development and maintenance of immune functions. Interleukin-1 (IL-1) is an important factor, secreted by

macrophages in response to immunogens such as lipopolysacharide (LPS). Histogranin per se does not induce the production of IL-1α mRNA nor does it increase the secretion of IL-1 in the extracellular compartment. LPS alone can induce a small production of IL-1α mRNA and a concomitant release of IL-1. However, the effects of LPS on the macrophage content of IL-1α mRNA and the secretion of IL-1 are markedly increased by histogranin [SEQ ID NO:1] and [Ser^1]histogranin [SEQ ID NO:2], starting at 10^{-9} M to reach a maximal response between 10^{-8} and 10^{-7} M. The marked potentiation of Il-1 production by histogranin in macrophages that are challenged with LPS indicates that histogranin can enhance the immune response in immune cells. Various pathophysiological conditions (AIDS, depression, stress, ultra-violet light and radiation exposures) are known to be accompanied by a decrease in immune functions. Peripheral injection of histogranin and histogranin agonists may then be of some beneficial value for the treatment of various types of immune disorders, the prime target for its enhancement of host-immune functions being circulating macrophages (a site readily accessible for peripherally administered peptides).

The invention is described in further experimental detail in the following Examples which are provided for the purpose of illustration only and should not be construed as limiting the scope of the invention.

EXAMPLE 1

Materials and Methods

Materials

The initial purification of histogranin (HN1) [SEQ ID NO:1] was achieved using an antibody raised against synthetic bombesin (Immunotech, Inc, MC). Specific antibodies were also prepared in rabbits against synthetic [Ser^1]-HN [SEQ ID NO:2] according to a procedure already described (Benoit et.al., Fed. Proc. 39, Abs 1166, 1980). [^{125}I][Tyr^4]-bombesin and [^{125}I]-[Ser^1]HN [SEQ ID NO:2] were prepared following the method of Hunter and Greenwood (Nature 258, 577, 1975) and purified by high performance liquid chrcmatography (HPLC) on a μ-Bondapak C18 column (3.9 x 300 mm; Waters). HPLC was run in 0.1% trifluoroacetic acid (TFA)/15% acetonitrile (ACN, initial condition) at 1 ml/min. The iodinated compounds were eluted with linear gradients of ACN (between 15% and 60%) within 50 min, starting at 10 min. Anti- Leu-Enkephalin (Leu-Enk) serum, synthetic [Tyr^4]-bombesin, neuromedin C, neuromedin B, Leu-Enk, vasoactive intestinal peptide (VIP), substance P, gastrin releasing peptide (GRP) and bombesin were purchased from Peninsula Inc. (Belmont, CA). Ethylenediamine-tetraacetic acid (EDTA), 2,2-thiodiethanol and TFA were products of Eastman Kodak (Rochester, NY).

Purification procedure

Two hundred bovine adrenal glands were collected at a local slaughterhouse and HN was purified according to a procedure already described for the extraction of neuromedin C (Lemaire et.al., Peptides, 10, 355, 1989). HN [SEQ ID NO:1] was coextracted and copurified with bombesin-like immunoreactive (BLI) peptides by passage of an acid extract of bovine adrenal medulla through SepPak C18 cartridges (Waters), HPLC on a preparative SP-5PW column (Waters), partition chromatography on Sephadex G-50 and reverse phase HPLC on a μ-Bondapak C18 column (Waters). The BLI material eluted between fractions 21 and 23 on μ-Bondapak C18 (Lemaire et.al., Peptides, 10, 355, 1989) was a peptide fraction that did not coelute with gastrin releasing peptide (GRP) or related

peptides such as neuromedin C and neuromedin B. It was collected, lyophilized and submitted to chromatography on an analytical SP-5PW column (3.9 x 130 mm, Waters) (Fig 2A). The chromatography was carried out at 1 ml per min starting with a solution of 0.01 M ammonium acetate (pH 4.5): 10% ACN. A gradient with respect to salt and pH was started at 10 min to reach 1 M ammonium acetate (pH 6.5): 10% ACN at 50 min. Two peaks of BLI peptide were monitored by radioimmunoassay (RIA), one coeluting with neuromedin C, another one eluting between neuromedin B and neuromedin C (Fig 2A). This latter BLI material (fractions 21 to 23) was collected and submitted to HPLC on a Zorbax ODS column (3.9 x 250 mm, Dupont, Fig 2B). The column was equilibrated with a solution of ACN (18%) in 0.1% TFA at 1 ml/min. The material was eluted with a linear gradient of ACN, starting at 10 min to reach 30% ACN in 0.1% TFA at 45 min. This chromatography gave a single peak of BLI (fractions 32-34), eluting between synthetic neuromedins C and B (Fig 2B). The corresponding fractions were collected, lyophilized and the recovered material was submitted to amino acid and sequence analysis.

Amino acid composition and sequence analysis

Amino acid analysis of acid digests was performed on a Picotag column (3.9 x 300 mm; Waters) after the derivatization of amino acids with phenylisothiocyanate (PITC) as described previously (Bidlinmeyer et.al., J. Chromatogr. 236, 93, 1984). Hydrolysis of the samples was carried out at 108°C for 24 hrs in 6 M HCl, 0.1% mercaptoethanol. Blanks were run by incubation of the acid solution in the absence of peptide material. Purified adrenal HN [SEQ ID NO:1] was also subjected to amino acid sequence analysis on a gas-phase sequenator (model 470A, Applied Biosystems) coupled in line to an HPLC analyzer. Sequence analysis was performed by Dr. M. Blum, Department of Biochemistry, University of Toronto, according to the procedure described by Henvick et.al., (J. Biol. Chem. 256, 7990, 1981).

Synthesis

The synthesis of HN [SEQ ID NO:1] and related peptides

was achieved by the solid-phase method (Merrifield R.B., J. Am. Chem. Soc. 85, 2149, 1963) according to a procedure already described (Lemaire et.al., Int. J. Peptide Protein Res. 27, 300, 1986; Turcotte et. al., Int. J. Peptide Protein Res., 23, 361-367, 1984). The completed peptides were cleaved from the resin with liquid HF at 0°C for 1 hr in the presence of 10% anisole and purified by gel filtration on Sephadex G-10 (2.5 x 35 cm column) and semi-preparative HPLC on Nucleosil C18 (30μ, 2.2 x 30 cm column). The HPLC chromatogram was run at pH 2.1 in 0.1% TFA and the peptide material was eluted with a gradient of ACN between 15% and 45% in 0.1% TFA within 60 min at 4 ml per min, starting at 10 min after the injection. The purity of the synthetic peptides was verified by thin layer chromatography on silica gel, analytical HPLC on μ-Bondapak C18, SP-5PW and Zorbax ODS columns, amino acid analysis and FAB mass spectrometry.

Radioimmunoassay

BLI peptides, immunoreactive (ir)-HN [SEQ ID NO:1] and ir-Leu-Enk were measured by RIA according to the method already described (Lemaire et.al., Regulatory Peptides 13, 133, 1986). The RIA were performed as follows: to duplicate polypropylene tubes were successively added 1) 10 μl of sample plus 90 μl of buffer A (0.15 M phosphate buffer, pH 7.4 containing 0.1% bovine serum albumin, 0.9% sodium chloride, 0.01% sodium azide), 2) 20,000 cpm of [^{125}I]-[Tyr^{4}]-bombesin or [^{125}I]-[Ser^{1}]HN [SEQ ID NO:2] or [^{125}I]-Leu-Enk in 100 μl of buffer A containing 0.05% poly-L-Lys and 3) 100 μl of 1/40,000, 1/5000 or 1/3000 dilutions of antiserum to synthetic bombesin or [Ser^{1}]HN [SEQ ID NO:2] or Leu-Enk, respectively. The limits of sensitivity of the assays for recognizing the presence of synthetic bombesin, HN [SEQ ID NO:1] and Leu-Enk in standard assays were 5, 10 and 30 fmol, respectively.

Subcellular and tissue distributions

The subcellular fractions of bovine adrenal medulla (nucleus, granules, mitochondria microsomes and cytosol) were prepared according to a modification (Schneider, Biochem. Pharmacol. 21, 2627, 1972) of the method of Winkler et. al.,

(Biochem. J. 118, 303, 1970) as described by Lemaire et. al. (Peptides 10, 355, 1989). HN [SEQ ID NO:1] was extracted by the addition of 10 vol (v/v) of acetic acid (1M), HCl (0.01N) and thiodiethanol (0.01%) and incubation at 95°C for 10 min. The extracts were homogenized and centrifuged at 28,000 x g for 30 min. The supernatants were lyophilized and passed through SepPak C18 cartridges and the peptide material was eluted with a solution (6 ml) of ACN 60% in 0.1% TFA. The eluted material was evaporated, lyophilized and submitted to analytical HPLC on μ-Bondapak C18 (Waters). Peptide material was eluted with a linear gradient of ACN between 18% (10 min) and 30% (30 min) in 0.1% TFA at 1 ml/min. Immunoreactive (ir)-HN [SEQ ID NO:1] and ir-Leu-Enk (Lemaire et.al., Can. J. Physiol. Pharmacol. 62, 484, 1984) were monitored in each fraction by RIA using the antibodies raised against synthetic [Ser^1]HN [SEQ ID NO:2] and Leu-Enk, respectively. The ir-peaks corresponding to the elution time of the synthetic peptides were integrated and results were expressed as pmol/mg protein (Lowry et.al., J. Biol. Chem. 193, 265, 1951).

Various tissues were also collected from Wistar rats (male, 200-400 g; Canadian Breeding Farm, St-Constant, Quebec). The tissues were minced and extracted with 10 vol of 1 M acetic acid-0.01M HCl-0.001% thiodiethanol at 95°C for 10 min. The extracts were allowed to cool on ice and homogenized with a glass teflon homogenizer. Homogenates were centrifuged at 46,000 x g for 30 min at 4°C and the supernatants were lyophilized, passed through SepPak C18 cartridges and a μ-Bondapak HPLC column as described above. Ir-HN [SEQ ID NO:1] was monitored in each fraction by RIA and the ir-peak coeluting with synthetic HN was integrated and results were expressed as pmol/g of tissue.

Secretion from perfused adrenal glands

Bovine adrenal glands, freshly obtained from a local slaughterhouse, were perfused at 10 ml/min in a retrograde manner with Krebs buffer at 37°C (Lemaire et al., Peptides, 10, 355, 1989). After a pre-equilibration period of 60 min, carbamylcholine (500 μM) was added to the perfusion buffer

during 1.5 min. The perfusate was collected 30 min before stimulation with carbamylcholine (control) and fractions of 5 min starting from the beginning of the stimulation were collected (up to 40 min). Fractions were combined and passed through SepPak C18 cartridges (Waters) and HPLC (μ-Bondapak) as described above before measurement of HN by RIA. The ir-peak coeluting with synthetic HN [SEQ ID NO:1] was integrated, collected, lyophilized and used for confirmation of the N-terminal sequence of natural HN after further purification on SP-5PW and Zorbax ODS HPLC columns as shown in Fig. 2 (see previous section on sequence determination).

Effect of histogranin on the binding of NMDA, PCP and σ receptor ligands

[^{3}H]CGP 39653 a specific NMDA receptor ligand, was used to monitor the interaction of histogranin with the NMDA receptor in rat brain membranes (Sills et al., Eur. J. Pharmacol. 192: 19, 1991). Typical binding assays were performed in 5 mM Tris-HCl buffer (pH 7.4) (buffer C) at 4°C for 60 min with 2 ml aliquots of the membrane preparation (0.4 mg protein/ml) in the presence of [^{3}H]CGP 39653 (5 nM) and various concentrations of histogranin, as indicated. Incubations were terminated by filtration under reduced pressure through GF934AH Whatman filters pretreated with 0.05% polyethylenimine. Filters were washed with 4 x 3-ml aliquots of ice cold buffer C, placed in liquid scintillation vials along with 10 ml Ecolume (ICN Biochemical Inc.) and counted in a Beckman liquid scintillation counter. Nonspecific binding of [^{3}H]CGP 39653 was determined in the presence of 10 μM CPP. Specific binding was defined as the difference between the radiolabel bound in the presence and absence of haloperidol. The concentration of HN that produces 50% inhibition of [^{3}H]CGP 39653 binding (IC_{50}) was derived using the nonlinear least square computer fitting program CDATA (EMF Software Inc., Knoxville, TN). The effect of histogranin on PCP and σ receptors were also monitored, using [^{3}H]MK-801 (5 nM) and [^{3}H](+)pentazocine (5 nM) as specific ligands, respectively; the binding experiments were performed as described above and the non specific binding was

determined in the presence of 10 μM of MK-801 and haloperidol, respectively.

Binding assays

Autoradiography of [^{125}I]-[Ser^{1}]-HN [SEQ ID NO:2] binding was performed according to the method described by Wolfe et.al. (Endocrinol. 124, 1160, 1989). Bovine adrenal tissue was sectioned at 10 μm and thaw-mounted onto chrome-alum/gelatin subbed microscopic slides (Canlab) and pre-incubated for 15 min at room temperature in buffer B (10mM Tris-HCl, pH 7.4). The solution was removed and the slides were incubated in buffer B (100 μl) supplemented with 0.5 nM [^{125}I]-[Ser^{1}]-HN [SEQ ID NO:2] for 1 hr at room temperature. Non-specific binding was determined in the presence of unlabelled HN [SEQ ID NO:1]. The binding was stopped by dipping the sections in cold buffer B in three consecutive baths (3 min each). They were then dipped rapidly in distilled water and dried under a stream of cold dry air. Dried sections were either counted directly by wiping the sections and counting them in 10 ml Aquasol in a Beckman apparatus or apposed to Hyperfilm (Amersham) and stored at -80°C for 3-5 days. After exposure, the autoradiograms were developed, fixed in Kodak Rapid fixer, examined and photographed.

[^{3}H]dextromethorphan binding was also carried out with membrane preparations of bovine adrenal medulla (Rogers et al Biochem.Pharmacol. 38, 2467, 1989) and rat brain (Lemaire et al Int. J. Peptide Protein Res. 27, 300, 1986). Typical binding assays were performed in 5 mM Tris-HCl buffer (pH 7.4) (buffer C) at room temperature (20-22° C) for 1 hr with 2 ml aliquots of the membrane preparation (0.5 mg protein/ml) in the presence of [^{3}H]dextromethorphan (5 nM) and various concentrations of HN [SEQ ID NO:1], as indicated. Incubations were terminated by placing the samples on ice followed by filtration under reduced pressure through GF/B Whatman filters pretreated with 0.05% polyethylenimine. Filters were washed with 4 x 3-ml aliquots of ice cold buffer C, placed in liquid scintillation vials along with 10 ml Aquasol (New England

Nuclear) and counted in a Beckman liquid scintillation counter. Nonspecific binding of [^{3}H]dextromethorphan was determined in the presence of 1 μM haloperidol. Specific binding was defined as the difference between the total radiolabel bound and that bound in the presence of haloperidol.

Anticonvulsive activity

Synthetic HN [SEQ ID NC:1] (10 nmol in 10 μl saline) was injected intracerebroventricularly (i.c.v.) in mice 5 min prior to injection of NMDA (0.25-1 nmol, i.c.v.). Control experiments were injected with saline (10 μl) 5 min prior to NMDA. NMDA alone produced convulsions that were characterized by treadle movements starting with one forelimb and spreading to the other forelimb and then to the hindlimbs. This progressed into running and jumping and in some cases, terminated in tonic hindlimb extension. At the end of convulsion episodes, the animals became immobile for 10 to 15 min. HN [SEQ ID NO:1] alone (10 nmol) produced no sign of convulsion. The potency of NMDA in producing convulsion in presence or absence of HN is expressed as the dose that induces 50% of the animals to convulse (AD_{50}). Groups of 10 animals were tested for each dose of NMDA.

Results

Purification of adrenomedullary histogranin

HN [SEQ ID NO:1] was purified from bovine adrenal medulla following a purification pattern designed for the isolation of BLI peptides (Lemaire et.al., Peptides, 10, 355, 1989). One of the BLI-peaks did not correspond to any known synthetic standards for the mammalian counterpart of bombesin (GRP, neuromedin B, neuromedin C). The unknown peptide eluted between the retention times of neuromedin B and neuromedin C on μ-Bondapak C18 (Lemaire et.al., Peptides, 10, 355, 1989), was purified by HPLC on SP-5PW (Fig. 2A) and Zorbax ODS (Fig. 2B). It was collected at each step of the purification and the last chromatography (Fig. 2B) allowed the separation of a pure peptide fraction (ca 200 pmol BLI).

Sequence determination and amino acid analysis

The sequence determination of the purified fraction was carried out with a 50 pmol sample by Edman degradation on a gas phase sequenator (BAS instrument). The sequence determination gave the following results in pmol per cycle: (cycle #1, Xxx); #2, Asn:6; #3, Tyr:5; #4, Ala:8; #5, Leu:6; #6, Lys:3; #7, Gly:7; #8, Gln:7; #9, Gly:6; #10, Arg:2; #11, Thr:4; #12, Leu:5; #13, Tyr:4; #14, Gly:2; #15, Phe:7. The identity of cycle #1 was not clear, being hindered by a high background (contamination with other amino acids). The first amino acid was identified as Met in three subsequent sequence analyses performed with the ir-material collected from the perfusate of carbamylcholine-stimulated adrenal glands (see the method section). Phe was assumed to be the C-terminal amino acid based on the amino acid analysis of the natural peptide: (Asp, 1.5 (1); Glu, 1.3 (1); Met, 0.8 (1); Gly, 2.8 (3); Thr, 1.0 (1); Ala, 0.7 (1); Arg, 1.0 (1); Tyr, 1.6 (2); Leu, 1.8 (2); Phe, 1.2 (1) and Lys, 0.8 (1)). Therefore, the sequence, H-Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH [SEQ ID NO:1], was more precisely designated as histogranin-(1-15) (in case that the natural peptide contains more than 15 amino acids). The name "histogranin" for this peptide was chosen partly due to the fact that its structure displayed 80% homology with that a 15 amino acid fragment (86 to 100) of histone H4 (-Val-Val-Tyr-Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe- [SEQ ID NO:72]; the underlined amino acids being common to both peptides). The only differences seen between the two structures reside in positions 1, 2 and 7 where Val, Val and Arg are replaced in HN [SEQ ID NO:1] by Met, Asn and Gly, respectively.

Synthesis of HN and related peptides

HN [SEQ ID NO:1], [Ser^1]HN [SEQ ID NO:2] and various analogues of HN were synthesized by the solid-phase procedure (Lemaire et al., Int. J. Peptide Protein Res., 27, 300, 1986) and purified on Sephadex G-10 and semi-preparative HPLC on Nucleosil C18. The purity and identify of the synthetic

peptides were verified by thin layer chromatography, analytical HPLC, amino acid analysis and FAB mass spectrometry. HN [SEQ ID NO:1] coeluted with the natural peptide in three HPLC systems with the following retention times: Zorbax ODS, 34.3 min; (Fig. 2B); μ-Bondapak, 22.0 min (Lemaire et al., Peptides, 10, 355, 1989), and SP-5PW, 22.0 min (Fig. 2A). Amino acid analysis of an acid digest of the synthetic peptide gave also the expected values for each amino acid: (Asp, 0.7; Glu, 0.8; Met, 1.1; Gly, 3.3; Thr, 1.0; Ala, 1.0; Arg, 1.2; Tyr, 2.2; Leu, 2.1; Phe, 1.1; Lys, 1.1). Synthetic HN [SEQ ID NO:1] was compared with other synthetic HN-related peptides on Zorbax ODS column. HN-(2-15) [SEQ ID NO:112], HN-(3-15) [SEQ ID NO:88], HN-Gly^{16}-Gly^{17} [SEQ ID NO:70] [(C-terminal portion of histone H4) and fragment-(86-100) [SEQ ID NO:72] of histone H4 gave retention times (35.3, 35.1, 30.7 and 39.0 min, respectively) that did not correspond to that of adrenomedullary HN [SEQ ID NO:1] (Fig. 2B; 34.3 min). Moreover, synthetic HN [SEQ ID NO:1] (10^{-10} - 10^{-9}M) displaced 20 % of the binding of [^{125}I][Tyr^4]bombesin to its antibody (the antiserum that was used for the detection and isolation of natural HN) while [Ser^1]HN [SEQ ID NO:2], HN-Gly^{16}-Gly^{17} [SEQ ID NO:70] and fragment-(86-100) of histone H4 [SEQ ID NO:72] had no cross-immunoreactivity, indicating that synthetic HN [SEQ ID NO:1] possessed a selective cross-immunoreactivity for anti-bombesin, a characteristic that was shared by natural HN [SEQ ID NO:1].

Characterization of rabbit anti-[Ser^1]histogranin

A rabbit antiserum was raised against [Ser^1]HN [SEQ ID NO:2], a stable analog of HN [SEQ ID NO:1], using the method of Benoit et.al., (Fed. Proc. Abs., 39, 1166, 1980). The purity of the synthetic peptide was assessed by HPLC on Zorbax ODS column (Fig. 3) and FAB mass spectrometry (Fig. 3 inset). The antiserum produced with this peptide had no cross-immunoreactivity with angiotensin and neurotensin. Its cross-reactivity to dynorphin B, dynorphin A, VIP, neuromedin C, substance P, GRP and bombesin was smaller than 0.01%. Its sensitivity to HN [SEQ ID NO:1] was identical to that of

[Ser^1]HN [SEQ ID NO:2] (30 fmol detection at the IC_{50}). The importance of the integrity of the C-terminal amino acids in HN [SEQ ID NO:1] for the immune reaction was indicated by its low cross-immunoreactivity with HN=Gly^{16}=Gly^{17} [SEQ ID NO:70] (0.14 %). On the other hand, the relative high immunoreactivity of HN-(2-15) [SEQ ID NO:113] and HN-(3-15) [SEQ ID NO:88] (17% and 23%, respectively) indicates that the presence of the N-terminal portion of the molecule is less important for the immune reaction.

Subcellular distribution of ir-histogranin

Subcellular fractions of bovine adrenal medulla (secretory granules, nucleus, mitochondria, microsomes and cytosol) were prepared and analyzed for their content in ir-HN [SEQ ID NO:1] and ir-Leu-Enk (Table 1). The subcellular fractions were analyzed by HPLC on μ-Bondapak C18 after acid extraction and passage through Sep Pak C18 (see the method section). HN [SEQ ID NO:1] and Leu-Enk were measured by RIA in each fraction and the ir-peaks corresponding to the retention time of the synthetic peptides were integrated. The secretory granules contained three major peaks of ir-HN, one of them (31 min) coeluting with the synthetic peptide (Fig. 4A). The other ir-peaks were eluted at longer pericds of time, possibly corresponding to some precursor forms of granule HN. The highest concentrations of ir-Leu-Enk and ir-HN [SEQ ID NO:1] were found in the secretory granules (Table 1). The nucleus contained very little ir-Leu-Enk (0.62 pmol/mg protein as compared with 16.9 pmol/mg protein in secretory granules) but its content in ir-HN [SEQ ID NO:1] (35 fmol/mg protein) was close to that found in the secretory granules (42 fmol/mg protein). The relative high level of ir-HN in the nuclear fraction may be relevant to the presence of histone H4 in this fraction which may cross-react with anti-HN. Mitochondria, microsomes and cytosol contained lower amounts of both peptides.

Secretion of ir-histogranin

Perfusates of bovine adrenal glands were analyzed for their content in ir-HN [SEQ ID NO:1] before (basal secretion) and upon stimulation with carbamylcholine. The basal secretion of ir-HN from perfused bovine adrenal glands averaged 6.5 ± 0.2 pmol/30 min/gland (n=6). Stimulation of the gland for 1.5 min in the presence of carbamylcholine (500μM) induced a large increase in the amount of ir-HN [SEQ ID NO:1] released during the first 40 min after stimulation (average of 110.5 ± 12 pmol/30 min/gland). HPLC analysis of the perfusates (Fig. 4B) indicated that the time of retention of ir-HN [SEQ ID NO:1] released upon stimulation with carbamylcholine corresponds to that of synthetic HN [SEQ ID NO:1]. Most of the secretion of HN in response to carbamylcholine occurred within 30 min, starting only 10 min after the beginning of the 1.5 min pulse-stimulation while the secretion of Leu-Enk was observed at the beginning (10 min) of the stimulation.

TABLE 1

Subcellular distribution of ir-Leu-Enk and ir-histogranin [SEQ ID NO:1] in extracts obtained from bovine adrenal medulla*.

Subcellular fraction	ir-Leu-Enk (pmol/mg prot)	ir-Leu-Enk Relative concentration**	ir-histogranin [SEQ ID NO:1] (fmol/mg prot)	ir-histogranin [SEQ ID NO:1] Relative concentration**
Secretory granules	16.9 ± 2.0	100	42 ± 3	100
Nucleus	0.6 ± 0.1	3	35 ± 4	83
Mitochondria	3.2 ± 0.5	18	25 ± 3	59
Microsomes	0.8 ± 0.1	4	16 ± 2	38
Cytosol	0.4 ± 0.1	2	1 ± 0.2	2

*Extracts of subcellular fractions and measurements were made as described under "Materials and Methods". Results are expressed as the mean ± SEM (n=3).

**Relative to secretory granules.

Tissue distribution of HN

Ir-HN [SEQ ID NO:1] was measured in extracts of various rat tissues (Table 2). The peptide was mostly concentrated in the pituitary (5,065 pmol/g) and the adrenal gland (268 pmol/g). Other important sources of the peptide were the spleen and the lung, two tissues that play an important role in host-defense immune responses. The brain and blood plasma contained low but significant levels of ir-HN [SEQ ID NO:1], with concentrations of 1.6 pmol/g and 24.0 fmol/ml, respectively. The heart, the liver and the kidney displayed levels that were comparable to that of the brain. It is believed that HN [SEQ ID NO:1] in the brain may be concentrated in very specific and specialized area but the localization of the peptide in the brain has not yet been investigated.

Table 2

Distribution of immunoreactive histogranin [SEQ ID NO:1] in plasma and various tissues of the rat.

Tissue	Histogranin* (pmol/g)	Histogranin* relative concentration (%)**
Pituitary	*5,065 ± 200*	*10*
Adrenal gland	*268 ± 40*	*5.3*
Lung	*14.9 ± 3.5*	*0.3*
Spleen	*12.3 ± 3.0*	*0.2*
Heart	*2.4 ± 0.6*	*0.05*
Kidney	*1.9 ± 0.5*	*0.04*
Liver	*1.8 ± 0.5*	*0.03*
Brain	*1.6 ± 0.4*	*0.03*
Plasma	*24.0 ± 5.0 fmol/ml*	-

* [SEQ ID NO:1] Results are expressed as the mean ± SEM of 3 preparations. Tissue and plasma extracts were prepared as described under "Materials and Methods".
**Relative to pituitary concentration.

Binding of [^{125}I]-[Ser^{1}]HN [SEQ ID NO:2]

The presence of HN [SEQ ID NO:1] in secretory granules indicated a possible neuropeptide function for this compound. One of the criteria that is normally assigned to a neuropeptide is its binding property to specific receptors located in the brain or in peripheral tissues that are innervated by the autonomic nervous system. The binding of [^{125}I]-[Ser^{1}]HN [SEQ ID NO:2] was assessed in tissue sections of bovine adrenal glands (Fig 5). Specific binding of the peptide was observed only in the medullary portion of the gland (Fig 5A). The binding was readily displaced by unlabelled HN [SEQ ID NO:1] (1 μM; Fig. 5B). The adrenal medulla is an organ that responds to stress by a discharge of its granule content of catecholamines in the circulation. The presence of the HN receptor on adrenal medullary cells indicates that HN may play a role in the response of the adrenal gland to stress. It may modulate its own release and/or the release of catecholamines.

Recently, the non-competitive NMDA receptor antagonist, [^{3}H]dextromethorphan was shown to bind with a high affinity to brain σ receptors and its binding was potentiated by the anticonvulsants phenytoin and ropizine (Walker et al., Pharmacol. Rev., 42, 355, 1990). The adrenal medulla was also shown to contain σ-like receptors (Rogers et al., Biochem. Pharmacol., 38, 2467, 1989); therefore, HN [SEQ ID NO:1] was herein tested for its putative ability to displace or enhance the binding of the σ receptor ligand, [^{3}H]dextromethorphan, to membrane preparations of bovine adrenal medulla and rat brain (Fig 6A and 6B). HN [SEQ ID NO:1] did not compete with the binding of [^{3}H]dextromethorphan but rather greatly potentiated its binding (up to 2 fold) with both membrane preparations. The half-maximal effects were observed at concentrations of HN [SEQ ID NO:1] between 10^{-8} and 10^{-7}M.

Specific interaction of histogranin with the NMDA receptor

Using membrane preparations of rat brain, histogranin was shown to displace the binding of the specific NMDA receptor ligand, [^{3}H]CGP 39653. The inhibition curve was shallow, displaying two phases, a high affinity phase with an IC_{50} of 0.6 nM and a low affinity phase with an IC_{50} of 3,955 nM. The low affinity phase represented the displacement of 67% of the binding sites as compared with 33% for the high affinity phase. Previous studies with rat brain membrane preparations have indicated that the NMDA receptor can be differentiated in two distinct components, one being modulated by PCP, the other one being insensitive to PCP (Yoneda and Ogita, J. Pharmacol. Exp. Ther. 259: 86, 1991). High concentrations (>10^{-6}M) of histogranin also displaced the binding of the PCP receptor ligand, [^{3}H]MK-801 (Fig. 13). Since the PCP receptor belongs to the NMDA receptor complex (Wong and Kemp, Annu. Rev. Pharmacol. Toxicol., 31: 401, 1991), a correlation may exist between the low affinity phase of the displacement of [^{3}H]CGP 39653 binding by histogranin and its effect on [^{3}H]MK-801 binding. On the other hand, the binding of the σ receptor ligand, [^{3}H](+)pentazocine was not affected by the presence of histogranin.

Blockade of NMDA-induced convulsions

NMDA (0.50 - 1 nmol, i.c.v. in 10 μl of saline) induced rapid and short lasting (1-2 min) convulsions in mice. Preinjection of HN [SEQ ID NO:1] (5-100 nmol/mouse, i.c.v.) dose-dependently blocked the convulsive activity of NMDA. The ED_{50} for NMDA increased from 0.61 nmol/mouse to 0.71, 0.81, 0.85 and 0.99 nmol/mouse after administration of 5, 10, 40 and 100 nmol of the peptide, respectively (Table 3). The potency ratios of NMDA in HN (10-100 nmol doses) pretreated animals were significantly decreased as compared with that of the saline pretreated group.

Table 3

Effect of histogranin on the potency of NMDA in inducing convulsion in mice.

Pretreatment	*NMDA (ED_{50} nmol/mouse) (95 % Confidence Limit)*	*Potency Ratio (95 % Confidence Limit)*
Saline	*0.61 (0.54 - 0.69)*	*1*
Histogranin (5 nmol/mouse)	*0.71 (0.61 - 0.83)*	*0.86 (0.83 - 0.88)*
Histogranin (10 nmol/mouse)	*0.81 (0.68 - 0.98)*	*0.75 (0.70 - 0.79)**
Histogranin (40 nmol/mouse)	*0.85 (0.68 - 1.07)*	*0.72 (0.64 - 0.79)**
Histogranin (100 nmol/mouse)	*0.99 (0.74 - 1.32)*	*0.62 (0.52 - 0.73)**

*P<0.05, compared to saline pretreatment. ED_{50}: dose that produced convulsion in 50 % of the animals. The data were analyzed by the Methods of Litchfield and Wilcoxon using the computer program of Tallarida and Murray (in "Pharmacologic calculation system, Pharm/PCS-Version 3, Life Science Associates, Bayport, N.Y., 1981).

EXAMPLE 2

Materials and Methods

Materials

[^{3}H]dextromethorphan (85.9 Ci/mmol) was a product of New England Nuclear, Boston, MA. PCP and 1-[1-(2-thienyl) cyclohexyl]piperidine (TCP) were gifts from Dr. H.W. Avdovich, Bureau of Drug Research, Ottawa, Canada. Haloperidol, captopril, bacitracin and spiperone were obtained from the Sigma chemical Co. (St.Louis, MD). (+)SKF-10047 [(+)-N-allylnormetazocine] was obtained from Natl. Inst. of Drug Abuse, Baltimore, MD, USA. (-)Butaclamol, (+)CPP [(+)-3-(2-carboxypiperazin-4 yl)propyl-1-phosphonic acid], NMDA, α-amino-5-methyl-4-isoxazoleproprionic acid (AMPA), kainate, bicuculline, (+)pentazocine and 3(+)PPP [R(+)-3-(3-hydroxyphenyl N-propylpiperidine hydrochloride] were purchased from Research Biochemicals, Natick, MA, USA. Bestatin and thiorphan were purchased from Peninsula Lab. Inc. (Belmont, CA). Chloromethylated polystyrene-divinylbenzene (1% cross linkage) was purchased from Pierce Chemical Company, Rockford, Illinois. Butyloxycarbonyl (Boc)-amino acids were purchased from Bachem Inc., Torrance, CA. Sephadex G-10 was obtained from Pharmacia Ltd., Montreal. Nucleosil C18 resin (15-30 μm) was a product of Macherey-Nagel Co., Duren, Germany. It was mounted in glass column (2.1 x 30 cm; Ace Glass Inc., Vineland, NJ). Hydrolysis of synthetic peptides was carried out in 6M HCl at 110°C for 24 h. Amino acid analysis of acid hydrolysates was performed by high performance liquid chromatography (HPLC) on a Pico-Tag column (0.3 x 25 cm; Waters) after derivatization of amino acids with phenylisothiocyanate as described by Bidlingmeyer et al., (J. Chromatogr., 336, 93, 1984). Thin layer chromatography of peptides was run on silica gel plates (0.2 mm, BDH Chemicals Associate of E. Merck, Downstad, Germany) with the following solvent system: BPAW, 1-butanol-pyridine-acetic acid-water (6:6:1.2:4.8). Peptides were detected with ninhydrin reagents.

Synthesis of [Ser^1]histogranin [SEQ ID NO:2] and fragments

[Ser^1]HN [SEQ ID NO:2] and related fragments were synthesized by the solid-phase procedure (Merrifield, J. Am. Chem. Soc. 85, 2149, 1963) as described previously (Lemaire et al., Int. J. Peptide Protein Res., 27, 300, 1986). The peptides were cleaved from the resin and deprotected with liquid HF at 0°C in the presence of anizole (10%, v/v). The synthetic compounds were then purified by chromatography on Sephadex G-10 (medium size) and HPLC on the semi-preparative Nucleosil C18 column. The products were eluted from the column with a gradient of acetonitrile (15% to 60%, starting at 10 min after injection at a flow rate of 4 ml/min within 60 min), collected (UV detection at 280 nm) and lyophilized to give a final recovery of 20-30% (based on the starting resin, substituted with 0.25-0.35 mmol of the C-terminal amino acid/g). The identity and purity of the synthetic peptides were verified by thin layer chromatography and analytical HPLC on μ-Bondapak C18 (Waters).

Binding assays

[^{125}I][Ser^1]HN [SEQ ID NO:2] was prepared following the method of Hunter and Greenwood (Nature, 194, 495, 1962) and purified by HPLC on a μ-Bondapak C18 column as described for the preparation of iodinated bombesin (Lemaire et al., Peptides, 10, 355, 1989). Rat brain membranes were prepared in 5 mM Tris HCl buffer (pH 7.4; buffer A) as described previously (Lemaire et al., Int. J. Peptide Protein Res., 27, 300, 1986). Binding of [^{125}I][Ser^1]HN [SEQ ID NO:2] (1000 Ci/mmol) was conducted with 0.5 ml of a diluted brain membrane preparation (1.0 mg protein/ml; Lowry et al., J. Biol. Chem., 193, 265, 1959) in the presence of bacitracin (25 μM), bestatin (30 μM), captopril (10 μM) and thiorphan (0.3 μM) and the indicated concentration of the iodinated peptide. The binding was performed on ice (0-4°C) for 45 min and stopped by filtration through 934-AH filters (Whatman) pretreated with 0.05% polyethylenimine. The filters were washed 4 times with 3 ml portions of an ice-cold solution of Tris-HCl (5 mM) containing polyethyleneglycol (PEG 8000; 6.6%) and counted in

a gamma counter (Beckman). The specific binding was calculated using the difference between the counts in the presence and absence of [Ser^1]HN [SEQ ID NO:2] (20 μM). K_D and B_{max} were derived by linear regression analysis of Scatchard plots of saturation binding experiments.

[^{3}H]dextromethorphan and [^{3}H]spermidine binding to membrane preparations of rat brain were performed with 1 mg of rat brain membranes in 2 ml of buffer A, at room temperature (22°C) for 30 min in the presence of 5 nM of the radiolabel and in the presence or absence of haloperidol (10^{-5}M) or spermidine (10^{-4}M), respectively. Specific binding was defined as the difference between the total radiolabel bound and that bound in the presence of haloperidol or spermidine. The potentiation of [^{3}H]dextromethorphan and [^{3}H]spermidine binding by [Ser^1]HN [SEQ ID NO:2] (10^{-10} - 5 x 10^{-5}M) was expressed as the percent increase over the basal binding in the absence of the peptide.

Behavioral assays

Male Swiss Webster [(SW)fBR] mice weighing 25-30 g (Canadian Breeding Farm, St-Constant, Que.) were housed five per cage in a room with controlled temperature (22 ± 2°C), humidity and artificial light (06.30 - 19.00 h). The animals had free access to food and water and were used after a minimum of four days of acclimation to housing conditions. NMDA, (0.25 - 1 nmol), AMPA (10-60 nmol) kainate (0.25-1 nmol) or bicuculline (1-7 nmol) were injected i.c.v. in 10 μl saline and the percent of animals displaying signs of convulsion was recorded. Convulsion was manifested by treadle movements starting with one forelimb and then spreading to the other forelimb and to the hindlimbs. Convulsions progressed into running and jumping and in some cases terminated in tonic hindlimb extension and immobility. The protective effect of [Ser^1]HN (10 or 50 nmol/mouse, icv) was verified by injection of the peptide 5 min prior to the administration of NMDA or other convulsive agents.

Results

Peptide synthesis

[Ser^1]HN [SEQ ID NO:2] was synthesized by the solid-phase technique and purified by gel filtration through Sephadex G-10 and semi-preparative HPLC on Nucleosil C18 resin as described by Turcotte et. al. (Int. J. Peptide Protein Res 23, 361-367, 1984). The purity of the synthetic peptide was assessed by analytical HPLC on μ-Bondapak C18, SP-5PW and Zorbax ODS columns (a single peak, 280 nm detection) as well as by thin layer chromatography (a single spot, ninhydrin detection). Amino acid analysis of an acid hydrolysate gave the expected amino acid ratios: Asp, 1.2; Glu, 1.3; Gly, 3.2; Ser, 1.1; Thr, 1.0; Ala, 1.0; Arg, 1.1; Tyr, 2.2; Leu, 1.8; Phe, 1.0 and Lys, 1.0. FAB mass spectrometry gave the expected signal corresponding to a molecular weight of 1675 (Fig 3, inset). HN [SEQ ID NO:1] and HN fragments were also synthesized and their purity were assessed by the same analytical criteria.

Selective receptor binding activity of [^{125}I][Ser^1]histogranin [SEQ ID NO:2]

[^{125}I][Ser^1]HN [SEQ ID NO:2] was tested for its ability to bind to specific receptors in membrane preparations of rat brain. The binding activity was proportional to the concentration of membrane proteins in the assay (between 0.1 and 1.0 mg protein per ml; Fig. 7). All consecutive binding assays were then conducted with 1 mg protein/ml. Saturation binding assays displayed a high affinity (K_D: 13.1 ± 1.0 nM) saturable (Bmax: 165 fmol ± 12 mg protein) binding site for [^{125}I][Ser^1]HN [SEQ ID NO:2] (Fig. 8). Analysis of the association and dissociation kinetics (Fig. 9) revealed that [^{125}I][Ser^1]HN [SEQ ID NO:2] associated rapidly to its receptor with a k_{+1} of 0.0549 min^{-1} while the dissociation had a k_{-1} of 0.508 min^{-1}. The overall calculation of $K_D = k_{-1}/k_{+1}$, revealed a K_D (9.25 nM) in agreement with that deducted from Scatchard plot analysis (Fig. 7; K_D: 13.1 nM).

The binding of [^{125}I][Ser^1]HN [SEQ ID NO:2] was readily displaced by HN [SEQ ID NO:1] (Ki: 72 nM), [Ser^1]HN [SEQ ID

NO:2] (Ki: 28 nM) and specific fragments of HN (Table 4). The fact that HN-(6-15) [SEQ ID NO:97] and [Ser^1]HN-(1-10) [SEQ ID NO:23] displayed significant binding potency while HN-(7-15) [SEQ ID NO:113] and HN-(8-15) [SEQ ID NO:114] were much less active indicates that the minimal active binding core resides inside fragment-(6-10). However, HN-(6-10) [SEQ ID NO:99] itself possessed a low (but significant) binding activity (0.01% as compared with [Ser^1]HN). Several other ligands (10^{-7} - 10^{-4}M) selective for PCP [PCP, TCP, MK-801], σ [haloperidol, (+)pentazocine, 3(+)PPP, dextromethorphan, (+)SKF-10047], dopamine [spiperone, GBR-12902], NMDA [CPP, NMDA] and opioid receptors [naloxone, met- and leu-enkephalins, dynorphin A] were inactive in displacing the binding of [Ser^1]HN [SEQ ID NO:2].

HN [SEQ ID NO:1] and spermidine binding sites

Spermidine is known to interact with the NMDA receptor complex (Fig. 1), potentiating the action of Glu and increasing the binding of [^{3}H]MK-801 (Williams et al., Life Sci, 48, 469-498, 1991). Among the various substances tested for competition with [^{125}][Ser^1]HN [SEQ ID NO:2] binding to rat brain membranes (Table 4), only peptides whose structure was similar or identical to that of HN [SEQ ID NO:1] displayed competition binding activity. However, spermidine was an effective blocker of [^{125}I][Ser^1]HN [SEQ ID NO:2] binding with an Ki of 20.0 μM (Table 4). In order to verify if the HN [SEQ ID NO:1] receptor was identical to the spermidine binding domain (Fig 1), [Ser^1]HN [SEQ ID NO:2] was tested for its ability to displace the binding of [^{3}H]spermidine (5 nM). [Ser^1]HN [SEQ ID NO:2] (10^{-9} - 10^{-5}M) did not inhibit but potentiated the binding of [^{3}H]spermidine (150 %) with an IC_{50} between 10^{-8} and 10^{-7}M (data not shown). This latter data indicates that even though HN and spermidine may both interact with the NMDA receptor complex (spermidine competes with HN binding), their sites of action are distinct (HN does not inhibit but potentiates spermidine binding) and their functions are opposite (HN blocks, spermidine potentiates NMDA receptor stimulation).

Table 4

Relative potency of [Ser^1]histogranin [SEQ ID NO:2], histogranin [SEQ ID NO:1] and fragments in displacing the binding of [^{125}I][Ser^1]histogranin [SEQ ID NO:2] to rat brain membranes

*Peptide	Ki (nM ± SD)	Relative potency (%)
[Ser^1]histogranin [SEQ ID NO:2]	28 ± 6.5	100
Histogranin [SEQ ID NO:1]	72 ± 8.3	39
Histogranin-(2-15) [SEQ ID NO:112]	1,365 ± 395	2.0
Histogranin-(3-15) [SEQ ID NO:88]	1,495 ± 534	1.9
Histogranin-(4-15) [SEQ ID NO:91]	6,641 ± 594	0.4
Histogranin-(5-15) [SEQ ID NO:94]	6,749 ± 487	0.4
Histogranin-(6-15) [SEQ ID NO:97]	6,864 ± 533	0.4
Histogranin-(7-15) [SEQ ID NO:113]	96,300 ± 6480	0.03
Histogranin-(8-15) [SEQ ID NO:114]	97,251 ± 4312	0.03
[Ser^1]Histogranin-(1-14) [SEQ ID NO:115]	1,160 ± 289	2.4
[Ser^1]Histogranin-(1-13) [SEQ ID NO:116]	1,278 ± 272	2.2
[Ser^1]Histogranin-(1-12) [SEQ ID NO:12]	1,821 ± 315	1.5
[Ser^1]Histogranin-(1-10) [SEQ ID NO:23]	5,977 ± 486	0.5
Histogranin-(6-10) [SEQ ID NO:99]	164,400 ± 12000	0.01
Spermidine	20,000 ± 1,000	0.1

Potentiation of [^{3}H]dextromethorphan binding activity

[^{3}H]dextromethorphan was previously shown to possess a high affinity for σ-like binding sites in brain membrane preparations (Tortella et al., 1989). However, the binding of [^{3}H]dextromethorphan was allosterically modified, by the presence of specific anticonvulsants such as ropizine and phenytoin, this effect resulting from an increase in the affinity of the receptor for [^{3}H]dextromethorphan (Walker et al., 1990). Herein, we have tested the ability of HN [SEQ ID

NO:1] and [Ser^1]HN [SEQ ID NO:2] to modify the binding of [^{3}H]dextromethorphan (5 nM) to membrane preparations of rat brain (Fig. 10A). Both peptides evoked a two step induction of [^{3}H]dextromethorphan binding, the first step (1.4 - 1.5 fold increase) occurring between 10^{-10} and 10^{-7} M, the second increase being observed at larger concentrations. The increase in [^{3}H]dextromethorphan binding activity induced by [Ser^1]HN [SEQ ID NO:1] was totally blocked by the presence of NMDA (100 μM), CPP (1 μM) and MK-801 (1 μM) but not of (+)pentazocine (1.0 μM) (Fig. 10B).

Behavioral effects of [Ser^1]histogranin [SEQ ID NO:2]

In mice, 10 and 50 nmol doses (i.c.v.) of [Ser^1]HN [SEQ ID NO:2] produced a brief increase in locomotion in some animals but when injected 5 min prior to NMDA (0.5, 0.7, 1.0 nmol, i.c.v.), it dose dependently blocked NMDA induced convulsions. The effective dose of NMDA that induced 50% of the mice to convulse (ED_{50}) was significantly increased in the presence of 10 and 50 nmol of [Ser^1]HN [SEQ ID NO:2] (from 0.58 nmole per mouse to 0.78 and .99 nmol per mouse, respectively; Table 5). The [Ser^1]HN [SEQ ID NO:2] fragments, [Ser^1]HN-(1-12) [SEQ ID NO:12] and [Ser^1]HN-(1-10) [SEQ ID NO:23] were slightly more potent than [Ser^1]HN [SEQ ID NO:2] in antagonizing NMDA-induced convulsion. [Ser^1]HN [SEQ ID NO:2] (10 nmol/mouse, i.c.v.) was also a potent blocker of AMPA-induced convulsion, the ED_{50} being increased from 16.65 to 41.19 nmol per mouse in the presence of the peptide. On the other hand, kainate-, bicuculline- and pentylene tetrazole-induced convulsions were not significantly affected by the presence of the peptide (no significant change in the potency ratios, Table 5).

Table 5

Effect of [Ser^1]histogranin and [Ser^1]histogranin-(1-12) on the potency of NMDA, AMPA, kainate and bicuculline in inducing convulsion in mice.

Treatment	*ED_{50} (nmol/mouse) (95 % Confidence Limit)*	*Potency Ratio (95 % Confidence Limit)*
NMDA-induced convulsion		
Saline	*0.58 (0.47 - 0.73)*	*1*
[Ser^1]histogranin (10 nmol)	*0.78 (0.67 - 0.90)*	*0.75 (0.58 - 0.98)**
[Ser^1]histogranin (50 nmol)	*0.99 (0.72 - 1.38)*	*0.56 (0.38 - 0.83)**
[Ser^1]HN-(1-12) (10 nmol)	*0.96 (0.66 - 1.39)*	*0.60 (0.71 - 0.52)**
[Ser^1]HN-(1-10) (10 nmol)	*0.89 (0.68 - 1.19)*	*0.66 (0.48 - 0.91)**
AMPA-induced convulsion		
Saline	*16.65 (11.31 - 24.51)*	*1*
[Ser^1]histogranin (10 nmol)	*41.19 (23.88 - 71.03)*	*0.40 (0.21 - 0.79)**
Kainate-induced convulsion		
Saline	*0.33 (0.24 - 0.45)*	*1*
[Ser^1]histogranin (10 nmol)	*0.57 (0.36 - 0.93)*	*0.57 (0.32 -1.01)*
Bicuculline-induced convulsion		
Saline	*2.68 (1.41 - 5.11)*	*1*
[Ser^1]histogranin (10 nmol)	*3.69 (1.95 - 6.99)*	*0.73 (0.29 - 1.80)*
Pentylene tetrazole-induced convulsion		
Saline	*2.37 (1.51 - 3.70)*	*1*
[Ser^1]histogranin (10 nmol)	*2.89 (1.95 - 4.29)*	*0.82 (0.77 - 0.86)*

*P<0.05 compared to saline. All compounds were injected i.c.v., the peptides (or saline) being administered 5 min prior to the convulsant. ED_{50} is the effective dose of NMDA, AMPA, kainate and bicuculline that produces convulsion in 50 % of the animals. Data were analyzed by the method of Litchfield and Wilcoxon using procedure 47 of the computer program of Tallarida and Murray.

EXAMPLE 3

Methods

Synthesis of histogranin and analogs

Histogranin (HN) and some of its analogs as indicated below were synthesized by the solid-phase procedure (Lemaire et al., Int. J. Peptide Protein Res. 27, 300;1986). The completed peptides were cleaved from the resin with liquid HF in the presence of anizole (10 %) at 0° for 1 hr and purified by passage through a column (2.5 x 35 cm) of Sephadex G-10 (medium size, Pharmacia) followed by semi-preparative HPLC on Nucleosil C18 (30 μ; 2.2 x 30 cm column). The synthetic peptides were eluted with a gradient of acetonitrile (ACN) (15-45% in 0.1% trifluoroacetic acid (TFA)).

The HN analogs prepared in this way were the following: [Ser^1]HN [SEQ ID NO:2], [Thr^1]HN [SEQ ID NO:3], [Gly^1]HN [SEQ ID NO:4], [Tyr^1]HN [SEQ ID NO:10], [Arg^1]HN [SEQ ID NO:34], [Ser^1, His^2]HN [SEQ ID NO:117], [Phe^1]HN [SEQ ID NO:9], [Val^1]HN [SEQ ID NO:7], [Gln^1]HN [SEQ ID NO:118], [Ala^1]HN [SEQ ID NO:6], [Ser^1]HN-Cys^{16} [SEQ ID NO:100], [Leu^1]HN [SEQ ID NO:5], [Ser^1, Arg^2]HN [SEQ ID NO:37], [Ser^1, Ala^2]HN [SEQ ID NO:119], [Ser^1, Ser^2]HN [SEQ ID NO:120], [Glu^1]HN [SEQ ID NO:121], [pGlu^1]HN [SEQ ID NO:127], [Val^1, Val^2, Arg^7]HN [SEQ ID NO:72], HN-(2-15) [SEQ ID NO:112], HN-(3-15) [SEQ ID NO:88], HN-(6-15) [SEQ ID NO:97], HN-(7-15) [SEQ ID NO:113], [Ser^1]HN-(1-10) [SEQ ID NO: 23], [Ser^1]HN-(1-12) [SEQ ID NO:12], [Ser^1]HN-(1-14) [SEQ ID NO:115], [Ser^1]HN-Gly^{16} [SEQ ID NO:122], [Ser^1]HN-Gly^{16}-Gly^{17} [SEQ ID NO:61] and [Ser^1]HN-amide [SEQ ID NO:2].

The purity and identity of the synthetic peptides were verified by thin layer chromatography on silica gel (butanol-pyridine-acetic acid-water; 6:6:1.2:4.8), analytical HPLC on μ-Bondapak C18 and amino acid analysis. The synthetic peptides eluted as single peaks on thin layer chromatography and on analytical HPLC; their amino acid composition corresponded to expected values.

Binding of histogranin.

[^{125}I][Ser^1]HN [SEQ ID NO:2] (1000 Ci/mmol) was prepared as described earlier (Lemaire et al., Peptides 10, 355, 1989) and

used for binding studies with membrane preparations of rat brain. Rat brain membranes (whole brain or isolated areas: hippocampus, cortex, striatum and cerebellum) were prepared in 50 mM Tris HCl buffer (pH 7.4) as described previously (Lemaire et al., Int. J. Peptide Protein res. 27, 300;1986). Binding was conducted with 0.5 ml of a membrane preparation (1.0 mg protein/ml) in the presence of bacitracin (25 μM), bestatin (30 μM), captopril (10 μM) and thiorphan (0.3 μM) and 0.25 nM of the iodinated peptide. The binding was performed on ice (0-4°) for 45 min and stopped by filtration through 934-AH filters (Whatman) pretreated with 0.05% polyethylenimine. The filters were washed 4 times with 3 ml portions of an ice-cold solution of Tris-HCl (50 mM) containing polyethyleneglycol (PEG 8000; 6.6 %) and counted in a γ-counter (Beckman). The specific binding was calculated using the difference between the counts in the presence and absence of [Ser^1]HN [SEQ ID NO:2] (20 μM).

Guinea pig ileum assay.

The guinea pig ileum (GPI) is known to contain PCP and sigma binding sites as well as the NMDA receptor (Shannon and Sawyer, J. Pharm. Exp. Ther. 251, 518-523, 1989). The depression of electrically-induced contractions of the GPI was measured as described by Kosterlitz and Waterfield (Ann. Rev. Pharmacol. 15, 29, 1975). The animals were sacrificed by decapitation. The ileum was rapidly dissected and mounted on a 10 ml double jacketed organ bath in Krebs solution (pH 7.4) at 37°C. Tension of 1 g was applied to the strip (1.5 cm long) and contractions were induced by electrical pulsations: 30 V, 10 ms delay, 0.1 Hz, 1 ms duration and monophasic. Dose-response inhibitions curves were constructed for the tested peptides and the concentration causing 50 % inhibition (IC_{50}) was determined by log-probit plots of six graded concentrations, each representing the mean ± s.e. of six different tissues.

Results

Histogranin binding site.

A high affinity (K_D: 13.1 ± 1 nM) saturable (Bmax: 165 ± 12 fmol/mg protein) binding site for [^{125}I][Ser^1]HN [SEQ ID NO:2] was previously described using membrane preparations of rat brain (Fig 8). The distribution of [^{125}I][Ser^1]HN [SEQ ID NO:2] (5 nM) binding in membrane preparations of various rat brain areas displayed a great similarity with that of [^{3}H]MK-801 (5 nM), the two receptors were mainly concentrated in the hippocampus and the cortex (Table 6). [^{3}H]MK-801 is a specific marker of the NMDA receptor complex, its binding site, the PCP binding domain, being located in the lumen of the NMDA linked ion channel (Fig. 1). The presence of high concentrations of the NMDA receptors in the hippocampus and cortex has also been observed by Yaneda and Ogita (J. Pharmac. Exp. Ther. 259, 86; 1990).

Structure-function relationships.

Competitive binding studies indicated that the ability of the various peptides in displacing [^{125}I][Ser^1]HN [SEQ ID NO:2] binding to membrane preparations of rat brain was structure related. [Ser^1]- [SEQ ID NO:2], [Thr^1]- [SEQ ID NO:3], [Arg^1]- [SEQ ID NO:34], [Phe^1]- [SEQ ID NO:9], [Tyr^1]- [SEQ ID NO:10], [Ala^1]- [SEQ ID NO:6], [Gly^1]- [SEQ ID NO:4] and [Val^1]-HN [SEQ ID NO:7] displayed a high affinity for the HN receptor (Table 7). Removal of the C- ([Ser^1]HN-(1-14) [SEQ ID NO:115]) or N- (HN-(2-15) [SEQ ID NO:112]) terminal amino acids induced a large decrease in the binding potency of the peptide (from 100% to 2.4 % and 1.5 % relative potency, respectively). The minimal active binding fragment resides within positions 6 and 10 of the molecule.

Table 6

Binding of [^{125}I][Ser^1]HN (0.5 nM) and [^{3}H]MK-801 (5 nM) to membrane preparations of whole rat brain and various brain areas.

Membrane preparation	[^{125}I][Ser^1]histogranin		[^{3}H]MK-801	
	fmol/mg protein*	Rel.binding (%)**	fmol/mg protein*	Rel.binding (%)**
Whole brain	15.8 ± 2.3	71	283 ± 27	39
Cortex	20.5 ± 4.1	93	635 ± 4	88
Striatum	10.9 ± 1.1	49	340 ± 21	47
Hippocampus	22.1 ± 4.2	100	718 ± 84	100
Cerebellum	12.9 ± 2.6	58	18 ± 4	3

*Results are expressed as the mean ± s.e.m. of three experiments performed in duplicate.
**Relative to hippocampus.

The potency of the various analogs and fragments of HN in displacing the binding of [^{125}I][Ser^1]HN [SEQ ID NO:2] to rat brain membranes was compared with their ability to inhibit the electrically-evoked contractions of the guinea pig ileum (GPI; Table 7). [Ser^1]HN [SEQ ID NO:2] itself was the most potent HN-related peptide tested in the GPI assay with an IC_{50} of 1.6 μM. The potency of the HN-related peptides in the GPI was dependent on the integrity of the N-terminal portion of the molecule. Removal of the N-terminal amino acid (HN-(2-15) [SEQ ID NO:112]) decreased the activity of the peptide down to 41 %, while removal of the two N-terminal amino acids (HN-(3-15) [SEQ ID NO:88]) destroyed all biological activity in the GPI assay. Replacement of the N-terminal amino acid by Thr [SEQ ID NO:3], Tyr [SEQ ID NO:10], Phe [SEQ ID NO:9], Leu [SEQ ID NO:5], Ala [SEQ ID NO:6], Gln [SEQ ID NO:118], Glu [SEQ ID NO:121], pGlu [SEQ ID NO:127] or Gly [SEQ ID NO:4], reduced the activity of the peptide on the GPI and binding assays, but the residual activity of these synthetic peptides indicate

that they may be considered as effective agonists. Position 2 can also be replaced by His [SEQ ID NO:117], Ala [SEQ ID NO:118] or Ser [SEQ ID NO:119] to provide agonists. Amidation of the C-terminal amino acid ([Ser^1]HN-amide [SEQ ID NO:2]) increased the potency of the peptide in the GPI (135% as compared with [Ser^1]HN [SEQ ID NO:2]). [Arg^1]HN [SEQ ID NO:34], [Ser^1, Arg^2]HN [SEQ ID NO:37], [Ser^1]HN-Gly^{16}-Gly^{17} [SEQ ID NO:61], [Ser^1]HN-Cys^{16} [SEQ ID NO:100], [Val^1, Val^2, Arg^7]HN [SEQ ID NO:72] [or fragment-(86-100) of histone H4] and HN-(3-15) [SEQ ID NO:88] did not display any potency on the GPI but their binding potency in the brain receptor assay was significant, indicating that these peptides are potential antagonists. Both activities reside within positions 1 and 10 of the peptide, but fragment-(1-12) is more potent in both assays. [Ser^1]HN-(1-12) [SEQ ID NO:12] was even more potent than [Ser^1]HN [SEQ ID NO:2] itself in the anticonvulsive assay (see previous data, Table 5). Fragment-(6-15) [SEQ ID NO:97] may be considered as a pure antagonist, not displaying any agonist activity in the GPI, but inhibiting the binding of [^{125}I][Ser^1]HN to rat brain membranes. Based on the above data and those listed in Table 7, it is proposed that the following changes in the structure of HN may lead to the production of useful compounds:

1) Small ligands: HN-(1-10) [SEQ ID NO:32], HN-(1-10)-amide [SEQ ID NO:32], HN-(6-10) [SEQ ID NO:99], HN-(6-10)-amide [SEQ ID NO:99], HN-(2-10) [SEQ ID NO:124], HN-(2-10)amide [SEQ ID NO:124], HN-(3-10) [SEQ ID NO:90], HN-(3-10)-amide [SEQ ID NO:90], HN-(4-10) [SEQ ID NO:93], HN-(4-10)-amide [SEQ ID NO:93], HN-(5-10) [SEQ ID NO:96], HN-(5-10)-amide [SEQ ID NO:96], HN-(6-12) [SEQ ID NO:98], HN-(6-12)-amide [SEQ ID NO:98].

2) Potent agonists: HN [SEQ ID NO:1], HN-amide [SEQ ID NO:1], [Ser^1]HN [SEQ ID NO:2], [Ser^1]HN-amide [SEQ ID NO:2], [Ser^1, His^2]HN [SEQ ID NO:117], [Ser^1, His^2]HN-amide [SEQ ID NO:117], [Ser^1]HN-Gly^{16} [SEQ ID NO:122], [Ser^1]HN-Gly^{16}-amide [SEQ ID NO:122], [Thr^1]HN [SEQ ID NO:13], [Thr^1]HN-amide [SEQ ID NO:13], [Tyr^1]HN [SEQ ID

NO:10], [Tyr^1]HN-amide [SEQ ID NO:10], [Phe^1]HN [SEQ ID NO:9], [Phe^1]HN-amide [SEQ ID NO:9], [Ala^1]HN [SEQ ID NO:6], [Ala^1]HN-amide [SEQ ID NO:6], [Gly^1]HN [SEQ ID NO:4], [Gly^1]HN-amide [SEQ ID NO:4], [pGlu^1]HN [SEQ ID NO:127], [pGlu^1]HN-amide [SEQ ID NO:127].

3) Potential antagonists: [Arg^1]HN [SEQ ID NO:34], [Arg^1]HN-amide [SEQ ID NO:34], [Arg^1]HN-(1-12) [SEQ ID NO:35], [Arg^1]HN-(1-12)-amide [SEQ ID NO:35], [Arg^2]HN [SEQ ID NO:125], [Arg^2]HN-amide [SEQ ID NO:125], [Ser^1, Arg^2]HN [SEQ ID NO:37], [Ser^1, Arg^2]HN-amide [SEQ ID NO:37], [Ser^1, Arg^2]HN-(1-12) [SEQ ID NO:38], [Ser^1, Arg^2]HN-(1-12)-amide [SEQ ID NO:38], HN-(3-15) [SEQ ID NO:88], HN-(3-15)-amide [SEQ ID NO:88], HN-(6-15) [SEQ ID NO:97], HN-(6-15)-amide [SEQ ID NO:97], [Ser^1]HN-Cys^{16}]HN [SEQ ID NO:100], [Ser^1]HN-Cys^{16}-amide [SEQ ID NO:100], [Ser^1]HN-Gly^{16}-Gly^{17} [SEQ ID NO:61], [Ser^1]HN-Gly^{16}-Gly^{17}-amide [SEQ ID NO:61], [Val^1, Val^2, Arg^7]HN [SEQ ID NO:72] (or fragment 86-100 of histone H4), [Val^1, Val^2, Lys^7]HN-amide [SEQ ID NO:111], [Lys^2]HN [SEQ ID NO:126], [Lys^2]HN-amide [SEQ ID NO:126].

Table 7

Comparison between the ability of various HN analogues in inhibiting the electrically evoked contractions of the guinea pig ileum (GPI) and competing with the binding of [^{125}I][Ser^1]HN to rat brain membranes.

Synthetic peptide[1]	GPI		[^{125}I][Ser^1]HN	
	IC_{50}(nM)	Rel.Pot.(%)	K_{i50} (nM)	Rel.Pot.(%)
[Ser^1]HN	1,641	100	28	100
[Thr^1]HN	1,893	87	193	15
[Gly^1]HN	6,318	26	144	19
[Tyr^1]HN	15,144	11	59	47
[Phe^1]HN	7,850	21	73	38
[Gln^1]HN	13,756	12	116	24
[Ala^1]HN	11,059	15	134	21
[Leu^1]HN	36,429	4	46	61
[Glu^1]HN	13,098	12	1,500	2
[p.Glu^1]HN	8,040	20	1,348	2
[Arg^1]HN	inactive[2]	-	55	51
[Ser^1]HN-Cys^{16}	inactive[2]	-	74	38
[Ser^1, His^2]HN	4,797	34	>10,000	<0.3
[Ser^1, Ala^2]HN	6,781	24	972	3
[Ser^1, Ser^2]HN	5,449	30	1,318	2.1
[Ser^1, Arg^2]HN	inactive[2]	-	8,474	0.3
[Val^1, Val^2, Arg^7]HN	inactive[2]	-	>10,000	<0.3
HN-(2-15)	3,947	41	1,810	1.5
HN-(3-15)	inactive[2]	-	1,495	1.8
HN-(4-15)	inactive[2]	-	6,641	0.4
HN-(6-15)	inactive[2,3]	-	6,864	0.4
HN-(7-15)	inactive[2]	-	96,300	0.03
HN-(6-10)	inactive[2]	-	164,400	0.02
[Ser^1]HN-(1-10)	54,000	3	6,000	0.5
[Ser^1]HN-(1-12)	27,051	6	1,821	1.5
[Ser^1]HN-(1-14)	-	-	1,160	2.4
[Ser^1]HN-Gly^{16}	2,983	55	5,778	0.4
[Ser^1]HN-Gly^{16}-Gly^{17}	inactive[2]	0	1,321	2.1
[Ser^1]HN-amide	1,212	135	155	18.1

[1]Note that [Ser^1]HN [SEQ ID NO:2], HN [SEQ ID NO:1], HN-(1-12) [SEQ ID NO:21] and HN-(2-15) [SEQ ID NO:112] (10nmol/mouse, i.c.v.) are potent blocker of NMDA induced convulsion (Table 5, data not shown). [2]Inactive at 10 μM. [3]HN-(6-15) antagonizes the effect of [Ser^1]HN on the GPI.

EXAMPLE 4

Materials and Methods

Animals

C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) were used at 6 to 8 wks old. Male Wistar rats weighing 250 - 300 g were purchased from Charles River Canada (St.Constant, Quebec). These animals were derived from a pathogen-free colony, shipped behind filter barriers and housed in isolated temperature-controlled quarters.

Materials

Rat IFN-γ (4 x 10^6 U per mg protein) was obtained from Holland Biotechnology. Lipopolysacharide (LPS, E. Coli 0127:B8) was purchased from Sigma Chemical Co. (St.Louis, MO). [Ser^1]histogranin ([Ser^1]HN) [SEQ ID NO:2] was synthesized in our laboratory by the solid-phase procedure as described previously (Lemaire et al., Int. J. Peptide Protein Res. 27, 300, 1986).

Alveolar macrophages

Alveolar macrophages (AM) were obtained by bronchoalveolar lavage of rats as described previously (Lemaire, I. et al. Am. J. Pathol. 122, 205; 1986). A total volume of 50 ml of sterile PBS (pH 7.4) was infused in 5 ml-aliquots in each animal. Lavage fluid recovery was 90 %. The bronchoalveolar cells were obtained by centrifugation at 200 x g at 4° C for 10 min, washed in PBS and finally resuspended in RPMI 1640 (GIBCO Laboratories, Grand Island, NY). Cells were counted in a haemocytometer chamber and viability was determined by trypan blue exclusion. Differential counts of lavage cells (Wright-Giemsa stain) indicated that 99 % of total bronchoalveolar cells were AM.

Culture of alveolar macrophages and stimulation

AM (0.5 x 10^6) were incubated in 1 ml of RPMI 1640 supplemented with 0.5 % FBS in 24-well microculture plates for 24 h in the presence or absence of LPS and [Ser^1]HN [SEQ ID NO:2] alone or in various combinations as indicated. After incubation, the cell suspensions were centrifuged and the cell-free supernatants and cell pellets collected and frozen at -80° C until they were assayed.

Interleukin-1 assay

D10 (N4)M cells (Hopkins and Humphreys, J. Immunol. Methods 120, 271-276, 1989) (10^4/well) were cultured in 96-well round-bottomed microculture plates in a final volume of 200 ul of complete medium (RPMI-1640) containing 10 % FBS, 5 x 10^{-5} M mercaptoethanol, Hepes (0.8 %), concanavalin A (Con A; 5 ug/ml) and recombinant IL-2 (30 U/ml) with serial dilutions of AM supernatants. Appropriate controls contained medium, IL-2, Con A, LPS, and [Ser^1]HN [SEQ ID NO:2] alone or in combination. Cultures in triplicate were incubated for 66 h at 37° C, pulsed with 1 uCi/well [^{3}H]thymidine (New England Nuclear, Dupont) and harvested at 72 h. Bioassay data were calculated as net cpm by the following formula: net cpm = (cpm of D10 cells + Con A + IL-2 + sample) - (cpm of D10 cells + Con A + IL-2). Incorporation of [^{3}H]thymidine in the presence of sample dilutions was compared with that in the presence of dilutions of a standard recombinant IL-1 preparation (Genzyme, 10^8 U/mg). IL-1 activity was quantified by calculation of half-maximal units and data are expressed as percent over control (in the presence of LPS).

Northern blot analysis of IL-1α mRNA

Total RNA was extracted from the AM cell pellets (after stimulation with LPS + [Ser^1]HN [SEQ ID NO:2]) by the procedure of Chomezinski and Sacchi (Anal. Biochem. 162, 156-159, 1987). RNA samples were frozen (-76° C) in 1 mM cDTA (pH 8.0)/0.5 % SDA. Formaldehyde-MOPS agarose minigel electrophoresis and Northern transfer to a Gelman Bio Trace RP charge modified Nylon 66 membrane were performed according to the method described by Fourney et al. (FOCUS 10, 5-7, 1988) except that the capillary transfer was made with Whatmann 3 MM. Following transfer, the membrane was baked for 2 h at 80° C, stored at room temperature between two sheets of Whatmann 3 MM paper and processed for hybridization with radiolabelled IL-1α cDNA within 72 h. Hybridization was allowed to proceed for 18 h at 42 ° C in presence of denatured radiolabelled probe (5 ng/ml). Membranes were washed sequentially for 2 x 20 min in 1 x SSC/0.5 % SDS at room temperature followed by 2 x 30 min

washes in 0.1 x 55 C/0.5 % SSC at 55° C. The membrane was kept moist and sealed in a polypropylene bag and subjected to autoradiography at -76° C for 2-5 days under Kodak XAR film using Dupont Cronnex Lightning plus intensifying screens. The spots of IL-1α mRNA were photographed.

Results

Effects of [Ser^1]HN on IL-1 production

Incubation of AM with LPS in the presence of various concentrations of [Ser^1]HN [SEQ ID NO:2] resulted in increases of IL-1 release (Fig 11). The maximal effect (1.85 fold stimulation) was observed at 10^{-7} M [Ser^1]HN [SEQ ID NO:2], but a significant increase was already observed at 10^{-9}M [Ser^1]HN [SEQ ID NO:2]. The peptide potentiated the release of IL-1 in response to LPS (5 ug/ml) but per se, it did not induce the release of IL-1 from AM. AM play a crucial role in lung defense and immunoregulation (Fels and Cohn, J. Appl. Physiol. 60, 353.1, 1986). Besides their role in phagocytosis and Ag presentation, AM have the ability to produce a variety of mediators, including IL-1 (Lemaire and Beaudoin, J. Leukocyte Biol. 36, 402, 1984). The modulation of AM IL-1 production by [Ser^1]HN may indicate that mammalian HN fulfils an important physiological role in the establishment and maintenance of immunocompetence (Krakauer, CRC Crit. Rev. L. Immunol. 6, 213, 1986).

Effect of [Ser^1]HN [SEQ ID NO:2] on IL-1α mRNA formation

In order to verify if the production of IL-1 in response to [Ser^1]HN [SEQ ID NO:2] is due to an increase in the translation of its mRNA and/or to an increase in the formation of IL-1 mRNA, AM were exposed to LPS in combination with increasing concentrations of [Ser^1]HN [SEQ ID NO:2] and their contents in IL-1α mRNA were measured (Fig 12). LPS alone was able to trigger the formation of low but significant levels of IL-1α mRNA (C: control) while [Ser^1]HN [SEQ ID NO:2] alone had no effect (not shown). Combinations of LPS (5 ug/ml) and various concentrations of the synthetic peptide produced

marked increases in the macrophages levels of IL-1α mRNA, the maximal effect being observed between 10^{-8} and 10^{-7} M of the peptide. We can then conclude that the increase in the amount of IL-1 secreted in response to the presence of [Ser^{1}]HN [SEQ ID NO:2] corresponds to an increase in the amount of mRNA. HN is a natural peptide that is being released from the pituitary and the adrenal medulla in response to stress. HN [SEQ ID NO:1] contained in lung and spleen in the vicinity (or microenvironment) of macrophages may also play an important role in the immune response.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(I) APPLICANT: Lemaire, Simon

(ii) TITLE OF INVENTION: Peptides Having Neuroprotective and Immunostimulatory Functions

(iii) NUMBER OF SEQUENCES: 127

(iv) CORRESPONDENCE ADDRESS:

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy Disk
(B) COMPUTER: IBM PC Compatible
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WordPerfect 5.1

(2) INFORMATION FOR SEQ ID NO:1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1

Met Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2

Ser Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:3

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids

(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3

Thr Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4

Gly Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5

Leu Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6

Ala Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7

Val Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

Ile Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

Phe Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10

Tyr Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11

Cys Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

Ser Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13

Thr Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

Gly Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15

Leu Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids

(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16

Ala Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17

Val Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18

Ile Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19

Phe Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:20

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20

Tyr Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR S. ID NO:21

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21

Met Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:22

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22

Cys Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:23

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23

Ser Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:24

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24

Thr Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:25

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25

Gly Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:26

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26

Leu Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:27

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27

Ala Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:28

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28

Val Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:29

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids

(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29

Ile Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:30

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30

Phe Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:31

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31

Tyr Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:32

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32

Met Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:33

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33

Cys Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:34

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34

Arg Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:35

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35

Arg Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:36

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36

Arg Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:37

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37

Ser Arg Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:38

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38

Ser Arg Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:39

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39

Ser Arg Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:40

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40

Ser Asn Tyr Ala Leu Lys Arg Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:41

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41

Ser Asn Tyr Ala Leu Lys Arg Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:42

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42

Ser Asn Tyr Ala Leu Lys Arg Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:43

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43

Arg Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
2 5 10 15

(2) INFORMATION FOR SEQ ID NO:44

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44

Arg Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
2 5 10

(2) INFORMATION FOR SEQ ID NO:45

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45

Arg Tyr Ala Leu Lys Gly Gln Gly Arg
2 5 10

(2) INFORMATION FOR SEQ ID NO:46

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46

Asn Tyr Ala Leu Lys Arg Gln Gly Arg Thr Leu Tyr Gly Phe
2 5 10 15

(2) INFORMATION FOR SEQ ID NO:47

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47

Asn Tyr Ala Leu Lys Arg Gln Gly Arg Thr Leu
2 5 10

(2) INFORMATION FOR SEQ ID NO:48

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48

Asn Tyr Ala Leu Lys Arg Gln Gly Arg
2 5 10

(2) INFORMATION FOR SEQ ID NO:49

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49

Tyr Ala Leu Arg Gly Gln Gly Arg Thr Leu Tyr Gly Phe
5 10 15

(2) INFORMATION FOR SEQ ID NO:50

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50

Tyr Ala Leu Arg Gly Gln Gly Arg Thr Leu
5 10

(2) INFORMATION FOR SEQ ID NO:51

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51

Tyr Ala Leu Arg Gly Gln Gly Arg
5 10

(2) INFORMATION FOR SEQ ID NO:52

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52

Ala Leu Arg Gly Gln Gly Arg Thr Leu Tyr Gly Phe
5 10 15

(2) INFORMATION FOR SEQ ID NO:53

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53

Ala Leu Arg Gly Gln Gly Arg Thr Leu
5 10

(2) INFORMATION FOR SEQ ID NO:54

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54

Ala Leu Arg Gly Gln Gly Arg
5 10

(2) INFORMATION FOR SEQ ID NO:55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55

Leu Arg Gly Gln Gly Arg Thr Leu Tyr Gly Phe
5 10 15

(2) INFORMATION FOR SEQ ID NO:56

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56

Leu Arg Gly Gln Gly Arg Thr Leu
5 10

(2) INFORMATION FOR SEQ ID NO:57

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57

Leu Arg Gly Gln Gly Arg
5 10

(2) INFORMATION FOR SEQ ID NO:58

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58

Arg Gly Gln Gly Arg Thr Leu Tyr Gly Phe
6 10 15

(2) INFORMATION FOR SEQ ID NO:59

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59

Arg Gly Gln Gly Arg Thr Leu
6 10

(2) INFORMATION FOR SEQ ID NO:60

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60

Arg Gly Gln Gly Arg
6 10

(2) INFORMATION FOR SEQ ID NO:61

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61

Ser Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:62

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62

Thr Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:63

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63

Gly Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:64

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64

Leu Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:65

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65

Ala Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:66

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66

Val Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:67

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67

Ile Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:68

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68

Phe Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:69

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69

Tyr Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:70

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70

Met Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:71

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71

Cys Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Gly Gly
17

(2) INFORMATION FOR SEQ ID NO:72

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72

Val Val Tyr Ala Leu Lys Arg Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:73

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73

Lys Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:74

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74

Lys Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:75

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75

Lys Asn Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:76

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76

Ser Lys Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:77

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77

Ser Lys Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:78

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78

Ser Lys Tyr Ala Leu Lys Gly Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:79

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79

Ser Asn Tyr Ala Leu Lys Lys Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:80

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80

Ser Asn Tyr Ala Leu Lys Lys Gln Gly Arg Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:81

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81

Ser Asn Tyr Ala Leu Lys Lys Gln Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:82

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82

Lys Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
5 10 15

(2) INFORMATION FOR SEQ ID NO:83

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83

Lys Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
5 10

(2) INFORMATION FOR SEQ ID NO:84

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84

Lys Tyr Ala Leu Lys Gly Gln Gly Arg
5 10

(2) INFORMATION FOR SEQ ID NO:85

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85

Asn Tyr Ala Leu Lys Lys Gln Gly Arg Thr Leu Tyr Gly Phe
5 10 15

(2) INFORMATION FOR SEQ ID NO:86

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86

Asn Tyr Ala Leu Lys Lys Gln Gly Arg Thr Leu
5 10

(2) INFORMATION FOR SEQ ID NO:87

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87

Asn Tyr Ala Leu Lys Lys Gln Gly Arg
5 10

(2) INFORMATION FOR SEQ ID NO:88

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88

Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
5 10 15

(2) INFORMATION FOR SEQ ID NO:89

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89

Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu
5 10

(2) INFORMATION FOR SEQ ID NO:90

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90

Tyr Ala Leu Lys Gly Gln Gly Arg
5 10

(2) INFORMATION FOR SEQ ID NO:91

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91

Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
5 10 15

(2) INFORMATION FOR SEQ ID NO:92

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92

Ala Leu Lys Gly Gln Gly Arg Thr Leu
5 10

(2) INFORMATION FOR SEQ ID NO:93

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93

Ala Leu Lys Gly Gln Gly Arg
5 10

(2) INFORMATION FOR SEQ ID NO:94

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94

Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
5 10 15

(2) INFORMATION FOR SEQ ID NO:95

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95

Leu Lys Gly Gln Gly Arg Thr Leu
5 10

(2) INFORMATION FOR SEQ ID NO:96

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96

Leu Lys Gly Gln Gly Arg
5 10

(2) INFORMATION FOR SEQ ID NO:97

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97

Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
10 15

(2) INFORMATION FOR SEQ ID NO:98

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98

Lys Gly Gln Gly Arg Thr Leu
10

(2) INFORMATION FOR SEQ ID NO:99

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99

Lys Gly Gln Gly Arg
10

(2) INFORMATION FOR SEQ ID NO:100

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100

Ser Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:101

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101

Thr Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:102

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102

Gly Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:103

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103

Leu Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:104

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104

Ala Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:105

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105

Val Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:106

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106

Ile Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:107

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107

Phe Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:108

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids

(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108

Tyr Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:109

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109

Met Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:110

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110

Cys Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

Cys
16

(2) INFORMATION FOR SEQ ID NO:111

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111

Val Val Tyr Ala Leu Lys Lys Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:112

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112

Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
5 10 15

(2) INFORMATION FOR SEQ ID NO:113

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113

Gly Gln Gly Arg Thr Leu Tyr Gly Phe
10 15

(2) INFORMATION FOR SEQ ID NO:114

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114

Gln Gly Arg Thr Leu Tyr Gly Phe
10 15

(2) INFORMATION FOR SEQ ID NO:115

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115

Ser Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:116

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116

Ser Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:117

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117

Ser His Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INORMATION FOR SEQ ID NO:118

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118

Gln Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:119

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119

Ser Ala Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:120

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids

(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120

Ser Ser Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:121

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121

Glu Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:122

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122

Ser Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15
Gly
16

(2) INFORMATION FOR SEQ ID NO:123

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123

Ser His Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:124

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124

Asn Tyr Ala Leu Lys Gly Gln Gly Arg
5 10

(2) INFORMATION FOR SEQ ID NO:125

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125

Met Arg Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:126

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126

Met Lys Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:127

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127

pGlu Asn Tyr Ala Leu Lys Gly Gln Gly Arg Thr Leu Tyr Gly Phe
1 5 10 15

What I claim is:

1. A peptide designated "histogranin-(1-15)" [SEQ ID NO:1] or "histogranin" having the structure:

H-Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH;

in substantially pure isolated form.

2. A peptide having a structure according to the following general formula:

$$H\text{-}R_1\text{-}Gln\text{-}Gly\text{-}Arg\text{-}R_2\text{-}CO\text{-}R_3$$

wherein:

R_1 represents one structure selected from the group consisting of:

X-Asn-Tyr-Ala-Leu-Lys-Gly (X being an hydroxyl-containing amino acid [Ser, Thr]);

Y-Asn-Tyr-Ala-Leu-Lys-Gly (Y being a hydrocarbon side chain-containing amino acid [Gly, Ala, Leu, Val, Ile]);

Z-Asn-Tyr-Ala-Leu-Lys-Gly (Z being an aromatic amino acid [Phe, Tyr]);

W-Asn-Tyr-Ala-Leu-Lys-Gly (W being a sulfur-containing amino acid [Met, Cys]);

A-Asn-Tyr-Ala-Leu-Lys-Gly; Ser-A-Tyr-Ala-Leu-Lys-Gly; Ser-Asn-Tyr-Ala-Leu-Lys-A; A-Tyr-Ala-Leu-Lys-Gly; Asn-Tyr-Ala-Leu-Lys-A; Tyr-Ala-Leu-A-Gly; Ala-Leu-A-Gly; Leu-A-Gly; A-Gly; and Val-Val-Tyr-Ala-Leu-Lys-A (A being a basic amino acid [Arg, Lys]);

R_2 represents one structure selected from the group consisting of:

a single covalent bond;

Thr-Leu;

Thr-Leu-Tyr-Gly-Phe;

Thr-Leu-Tyr-Gly-Phe-Cys; and

Thr-Leu-Tyr-Gly-Phe-Gly-Gly;

and R_3 represents a radical selected from the group

consisting of -OH and $-NH_2$.

3. A peptide according to claim 2, designated [X^1]histogranin, having the structure: H-X-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein X is an hydroxyl-containing amino acid.

4. A peptide according to claim 2, designated [Y^1]histogranin, having the structure: H-Y-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein Y is a hydrocarbon side chain-containing amino acid.

5. A peptide according to claim 2, designated [Z^1]histogranin, having the structure: H-Z-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein Z is an aromatic amino acid.

6. A peptide according to claim 2, designated [W^1]histogranin, having the structure: H-W-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein W is a sulfur-containing amino acid.

7. A peptide according to claim 2, designated [X^1]histogranin-(1-12), having the structure: H-X-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein X is an hydroxyl-containing amino acid.

8. A peptide according to claim 2, designated [Y^1]histogranin-(1-12), having the structure: H-Y-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein Y is a hydrocarbon side chain-containing amino acid.

9. A peptide according to claim 2, designated [Z^1]histogranin-(1-12), having the structure: H-Z-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein Z is an aromatic amino acid.

10. A peptide according to claim 2, designated [W^1]histogranin-(1-12), having the structure: H-W-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein W is a sulfur-containing amino acid.

11. A peptide according to claim 2, designated [X^1]histogranin-(1-10), having the structure: H-X-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-COOH; wherein X is an hydroxyl-containing amino acid.

12. A peptide according to claim 2, designated [Y^1]histogranin-(1-10), having the structure: H-Y-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-COOH; wherein Y is a hydrocarbon side chain-containing amino acid.

13. A peptide according to claim 2, designated [Z^1]histogranin-(1-10), having the structure: H-Z-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-COOH; wherein Z is an aromatic amino acid.

14. A peptide according to claim 2, designated [W^1]histogranin-(1-10), having the structure: H-W-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-COOH; wherein W is a sulfur-containing amino acid.

15. A peptide according to claim 2, designated [A^1]histogranin, having the following structure: H-A-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

16. A peptide according to claim 2, designated [A^1]histogranin-(1-12), having the structure H-A-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein A is a basic amino acid.

17. A peptide according to claim 2, designated [A^1]histogranin-(1-10), having the structure H-A-Asn-Tyr-Ala-

Leu-Lys-Gly-Gln-Gly-Arg-COOH; wherein A is a basic amino acid.

18. A peptide according to claim 2, designated [Ser^1, A^2]histogranin, having the following structure: H-Ser-A-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

19. A peptide according to claim 2, designated [Ser^1, A^2]histogranin-(1-12), having the structure H-Ser-A-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein A is a basic amino acid.

20. A peptide according to claim 2, designated [Ser^1, A^2]histogranin-(1-10), having the structure H-Ser-A-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-COOH; wherein A is a basic amino acid.

21. A peptide according to claim 2, designated [Ser^1,A^7]histogranin, having the following structure: H-Ser-Asn-Tyr-Ala-Leu-Lys-A-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

22. A peptide according to claim 2, designated [Ser^1, A^7]histogranin-(1-12), having the following structure: H-Ser-Asn-Tyr-Ala-Leu-Lys-A-Gln-Gly-Arg-Thr-Leu-COOH; wherein A is a basic amino acid.

23. A peptide according to claim 2, designated [Ser^1, A^7]histogranin-(1-10), having the following structure: H-Ser-Asn-Tyr-Ala-Leu-Lys-A-Gln-Gly-Arg-COOH; wherein A is a basic amino acid.

24. A peptide according to claim 2, designated [A^2]histogranin-(2-15), having the following structure: H-A-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

25. A peptide according to claim 2, designated

[A^2]histogranin-(2-12), having the following structure: H-A-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein A is a basic amino acid.

26. A peptide according to claim 2, designated [A^2]histogranin-(2-10), having the following structure: H-A-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-COOH; wherein A is a basic amino acid.

27. A peptide according to claim 2, designated [A^7]histogranin (2-15), having the following structure: H-Asn-Tyr-Ala-Leu-Lys-A-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

28. A peptide according to claim 2, designated [A^7]histogranin-(2-12), having the following structure H-Asn-Tyr-Ala-Leu-Lys-A-Gln-Gly-Arg-Thr-Leu-COOH; wherein A is a basic amino acid.

29. A peptide according to claim 2, designated [A^7]histogranin-(2-10), having the following structure H-Asn-Tyr-Ala-Leu-Lys-A-Gln-Gly-Arg-COOH; wherein A is a basic amino acid.

30. A peptide according to claim 2, designated [A^6]histogranin-(3-15), having the structure: H-Tyr-Ala-Leu-A-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

31. A peptide according to claim 2, designated [A^6]histogranin-(3-12), having the structure H-Tyr-Ala-Leu-A-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein A is a basic amino acid.

32. A peptide according to claim 2, designated [A^6]histogranin-(3-10), having the structure H-Tyr-Ala-Leu-A-Gly-Gln-Gly-Arg-COOH; wherein A is a basic amino acid.

33. A peptide according to claim 2, designated [A^6]histogranin-(4-15), having the structure H-Ala-Leu-A-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

34. A peptide according to claim 2, designated [A^6]histogranin-(4-12), having the structure H-Ala-Leu-A-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein A is a basic amino acid.

35. A peptide according to claim 2, designated [A^6]histogranin-(4-10), having the structure H-Ala-Leu-A-Gly-Gln-Gly-Arg-COOH; wherein A is a basic amino acid.

36. A peptide according to claim 2, designated [A^6]histogranin-(5-15), having the structure H-Leu-A-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

37. A peptide according to claim 2, designated [A^6]histogranin-(5-12), having the structure H-Leu-A-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein A is a basic amino acid.

38. A peptide according to claim 2, designated [A^6]histogranin-(5-10), having the structure H-Leu-A-Gly-Gln-Gly-Arg-COOH; wherein A is a basic amino acid.

39. A peptide according to claim 2, designated [A^6]histogranin-(6-15), having the structure H-A-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

40. A peptide according to claim 2, designated [A^6]histogranin-(6-12), having the following structure: H-A-Gly-Gln-Gly-Arg-Thr-Leu-COOH; wherein A is a basic amino acid.

41. A peptide according to claim 2, designated [A^6]histogranin-(6-10), having the following structure: H-A-Gly-Gln-Gly-Arg-COOH; wherein A is a basic amino acid.

42. A peptide according to claim 2, designated [X^1]histogranin-Gly^{16}-Gly^{17}, having the structure: H-X-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly-COOH; wherein X is an hydroxyl-containing amino acid.

43. A peptide according to claim 2, designated [Y^1]histogranin-Gly^{16}-Gly^{17}, having the structure: H-Y-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly-COOH; wherein Y is a hydrocarbon side chain-containing amino acid.

44. A peptide according to claim 2, designated [Z^1]histogranin-Gly^{16}-Gly^{17}, having the structure: H-Z-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly-COOH; wherein Z is an aromatic amino acid.

45. A peptide according to claim 2, designated [W^1]histogranin-Gly^{16}-Gly^{17}, having the structure: H-W-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly-COOH; wherein W is a sulfur-containing amino acid.

46. A peptide according to claim 2, designated [X^1]histogranin-Cys^{16}, having the structure: H-X-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Cys-COOH; wherein X is an hydroxyl-containing amino acid.

47. A peptide according to claim 2, designated [Y^1]histogranin-Cys^{16}, having the structure: H-Y-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Cys-COOH; wherein Y is a hydrocarbon side chain-containing amino acid.

48. A peptide according to claim 2, designated [Z^1]histogranin-Cys^{16}, having the structure: H-Z-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Cys-COOH; wherein Z is an aromatic amino acid.

49. A peptide according to claim 2, designated [W^1]histogranin-Cys^{16}, having the structure: H-W-Asn-Tyr-Ala-

Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Cys-COOH; wherein W is a sulfur-containing amino acid.

50. A peptide according to claim 2, designated [Val^1, Val^2, A^7]histogranin, having the structure H-Val-Val-Tyr-Ala-Leu-Lys-A-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-COOH; wherein A is a basic amino acid.

51. A C-terminal amidated ($-CONH_2$) form of histogranin or of histogranin analogs as defined in any one of claims 3 to 48.

52. A peptide according to claim 2, said peptide being selected from the group consisting of:

HN	[SEQ ID NO:1]
HN-amide	[SEQ ID NO:1]
[Ser^1]HN	[SEQ ID NO:2]
[Ser^1]HN-amide	[SEQ ID NO:2]
[Thr^1]HN	[SEQ ID NO:3]
[Thr^1]HN-amide	[SEQ ID NO:3]
[Gly^1]HN	[SEQ ID NO:4]
[Gly^1]HN-amide	[SEQ ID NO:4]
[Leu^1]HN	[SEQ ID NO:5]
[Leu^1]HN-amide	[SEQ ID NO:5]
[Ala^1]HN	[SEQ ID NO:6]
[Ala^1]HN-amide	[SEQ ID NO:6]
[Val^1]HN	[SEQ ID NO:7]
[Val^1]HN-amide	[SEQ ID NO:7]
[Ile^1]HN	[SEQ ID NO:8]
[Ile^1]HN-amide	[SEQ ID NO:8]
[Phe^1]HN	[SEQ ID NO:9]
[Phe^1]HN-amide	[SEQ ID NO:9]
[Tyr^1]HN	[SEQ ID NO:10]
[Tyr^1]HN-amide	[SEQ ID NO:10]
[Cys^1]HN	[SEQ ID NO:11]
[Cys^1]HN-amide	[SEQ ID NO:11]
[Ser^1]HN-(1-12)	[SEQ ID NO:12]
[Ser^1]HN-(1-12)-amide	[SEQ ID NO:12]

[Thr^1]HN-(1-12)	[SEQ ID NO:13]
[Thr^1]HN-(1-12)-amide	[SEQ ID NO:13]
[Gly^1]HN-(1-12)	[SEQ ID NO:14]
[Gly^1]HN-(1-12)-amide	[SEQ ID NO:14]
[Leu^1]HN-(1-12)	[SEQ ID NO:15]
[Leu^1]HN-(1-12)-amide	[SEQ ID NO:15]
[Ala^1]HN-(1-12)	[SEQ ID NO:16]
[Ala^1]HN-(1-12)-amide	[SEQ ID NO:16]
[Val^1]HN-(1-12)	[SEQ ID NO:17]
[Val^1]HN-(1-12)-amide	[SEQ ID NO:17]
[Ile^1]HN-(1-12)	[SEQ ID NO:18]
[Ile^1]HN-(1-12)-amide	[SEQ ID NO:18]
[Phe^1]HN-(1-12)	[SEQ ID NO:19]
[Phe^1]HN-(1-12)-amide	[SEQ ID NO:19]
[Tyr^1]HN-(1-12)	[SEQ ID NO:20]
[Tyr^1]HN-(1-12)-amide	[SEQ ID NO:20]
HN-(1-12)	[SEQ ID NO:21]
HN-(1-12)-amide	[SEQ ID NO:21]
[Cys^1]HN-(1-12)	[SEQ ID NO:22]
[Cys^1]HN-(1-12)-amide	[SEQ ID NO:22]
[Ser^1]HN-(1-10)	[SEQ ID NO:23]
[Ser^1]HN-(1-10)-amide	[SEQ ID NO:23]
[Thr^1]HN-(1-10)	[SEQ ID NO:24]
[Thr^1]HN-(1-10)-amide	[SEQ ID NO:24]
[Gly^1]HN-(1-10)	[SEQ ID NO:25]
[Gly^1]HN-(1-10)-amide	[SEQ ID NO:25]
[Leu^1]HN-(1-10)	[SEQ ID NO:26]
[Leu^1]HN-(1-10)-amide	[SEQ ID NO:26]
[Ala^1]HN-(1-10)	[SEQ ID NO:27]
[Ala^1]HN-(1-10)-amide	[SEQ ID NO:27]
[Val^1]HN-(1-10)	[SEQ ID NO:28]
[Val^1]HN-(1-10)-amide	[SEQ ID NO:28]
[Ile^1]HN-(1-10)	[SEQ ID NO:29]
[Ile^1]HN-(1-10)-amide	[SEQ ID NO:29]
[Phe^1]HN-(1-10)	[SEQ ID NO:30]
[Phe^1]HN-(1-10)-amide	[SEQ ID NO:30]
[Tyr^1]HN-(1-10)	[SEQ ID NO:31]

[Tyr^1]HN-(1-10)-amide [SEQ ID NO:31]
HN-(1-10) [SEQ ID NO:32]
HN-(1-10)-amide [SEQ ID NO:32]
[Cys^1]HN-(1-10) [SEQ ID NO:33] and
[Cys^1]HN-(1-10)-amide [SEQ ID NO:33].

53. A peptide according to claim 2, said peptide being selected from the group consisting of:

[Arg^1]HN [SEQ ID NO:34]
[Arg^1]HN-amide [SEQ ID NO:34]
[Arg^1]HN-(1-12) [SEQ ID NO:35]
[Arg^1]HN-(1-12)-amide [SEQ ID NO:35]
[Arg^1]HN-(1-10) [SEQ ID NO:36]
[Arg^1]HN-(1-10)-amide [SEQ ID NO:36]
[Ser^1, Arg^2]HN [SEQ ID NO:37]
[Ser^1, Arg^2]HN-amide [SEQ ID NO:37]
[Ser^1, Arg^2]HN-(1-12) [SEQ ID NO:38]
[Ser^1, Arg^2]HN-(1-12)-amide [SEQ ID NO:38]
[Ser^1, Arg^2]HN-(1-10) [SEQ ID NO:39]
[Ser^1, Arg^2]HN-(1-10)-amide [SEQ ID NO:39]
[Ser^1, Arg^7]HN [SEQ ID NO:40]
[Ser^1, Arg^7]HN-amide [SEQ ID NO:40]
[Ser^1, Arg^7]HN-(1-12) [SEQ ID NO:41]
[Ser^1, Arg^7]HN-(1-12)-amide [SEQ ID NO:41]
[Ser^1, Arg^7]HN-(1-10) [SEQ ID NO:42]
[Ser^1, Arg^7]HN-(1-10)-amide [SEQ ID NO:42]
[Arg^2]HN-(2-15) [SEQ ID NO:43]
[Arg^2]HN-(2-15)-amide [SEQ ID NO:43]
[Arg^2]HN-(2-12) [SEQ ID NO:44]
[Arg^2]HN-(2-12)-amide [SEQ ID NO:44]
[Arg^2]HN-(2-10) [SEQ ID NO:45]
[Arg^2]HN-(2-10)-amide [SEQ ID NO:45]
[Arg^7]HN-(2-15) [SEQ ID NO:46]
[Arg^7]HN-(2-15)-amide [SEQ ID NO:46]
[Arg^7]HN-(2-12) [SEQ ID NO:47]
[Arg^7]HN-(2-12)-amide [SEQ ID NO:47]
[Arg^7]HN-(2-10) [SEQ ID NO:48]

[Arg^{7}]HN-(2-10)-amide	[SEQ ID NO:48]
[Arg^{6}]HN-(3-15)	[SEQ ID NO:49]
[Arg^{6}]HN-(3-15)-amide	[SEQ ID NO:49]
[Arg^{6}]HN-(3-12)	[SEQ ID NO:50]
[Arg^{6}]HN-(3-12)-amide	[SEQ ID NO:50]
[Arg^{6}]HN-(3-10)	[SEQ ID NO:51]
[Arg^{6}]HN-(3-10)-amide	[SEQ ID NO:51]
[Arg^{6}]HN-(4-15)	[SEQ ID NO:52]
[Arg^{6}]HN-(4-15)-amide	[SEQ ID NO:52]
[Arg^{6}]HN-(4-12)	[SEQ ID NO:53]
[Arg^{6}]HN-(4-12)-amide	[SEQ ID NO:53]
[Arg^{6}]HN-(4-10)	[SEQ ID NO:54]
[Arg^{6}]HN-(4-10)-amide	[SEQ ID NO:54]
[Arg^{6}]HN-(5-15)	[SEQ ID NO:55]
[Arg^{6}]HN-(5-15)-amide	[SEQ ID NO:55]
[Arg^{6}]HN-(5-12)	[SEQ ID NO:56]
[Arg^{6}]HN-(5-12)-amide	[SEQ ID NO:56]
[Arg^{6}]HN-(5-10)	[SEQ ID NO:57]
[Arg^{6}]HN-(5-10)-amide	[SEQ ID NO:57]
[Arg^{6}]HN-(6-15)	[SEQ ID NO:58]
[Arg^{6}]HN-(6-15)-amide	[SEQ ID NO:58]
[Arg^{6}]HN-(6-12)	[SEQ ID NO:59]
[Arg^{6}]HN-(6-12)-amide	[SEQ ID NO:59]
[Arg^{6}]HN-(6-10)	[SEQ ID NO:60]
[Arg^{6}]HN-(6-10)-amide	[SEQ ID NO:60]
[Ser^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:61]
[Ser^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:61]
[Thr^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:62]
[Thr^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:62]
[Gly^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:63]
[Gly^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:63]
[Leu^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:64]
[Leu^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:64]
[Ala^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:65]
[Ala^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:65]
[Val^{1}]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:66]
[Val^{1}]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:66]

[Ile^1]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:67]
[Ile^1]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:67]
[Phe^1]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:68]
[Phe^1]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:68]
[Tyr^1]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:69]
[Tyr^1]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:69]
HN-Gly^{16}-Gly^{17}	[SEQ ID NO:70]
HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:70]
[Cys^1]HN-Gly^{16}-Gly^{17}	[SEQ ID NO:71]
[Cys^1]HN-Gly^{16}-Gly^{17}-amide	[SEQ ID NO:71]
[Val^1, Val^2, Arg^7]HN	[SEQ ID NO:72]
[Val^1, Val^2, Arg^7]HN-amide	[SEQ ID NO:72]
[Lys^1]HN	[SEQ ID NO:73]
[Lys^1]HN-amide	[SEQ ID NO:73]
[Lys^1]HN-(1-12)	[SEQ ID NO:74]
[Lys^1]HN-(1-12)-amide	[SEQ ID NO:74]
[Lys^1]HN-(1-10)	[SEQ ID NO:75]
[Lys^1]HN-(1-10)-amide	[SEQ ID NO:75]
[Ser^1, Lys^2]HN	[SEQ ID NO:76]
[Ser^1, Lys^2]HN-amide	[SEQ ID NO:76]
[Ser^1, Lys^2]HN-(1-12)	[SEQ ID NO:77]
[Ser^1, Lys^2]HN-(1-12)-amide	[SEQ ID NO:77]
[Ser^1, Lys^2]HN-(1-10)	[SEQ ID NO:78]
[Ser^1, Lys^2]HN-(1-10)-amide	[SEQ ID NO:78]
[Ser^1, Lys^7]HN	[SEQ ID NO:79]
[Ser^1, Lys^7]HN-amide	[SEQ ID NO:79]
[Ser^1, Lys^7]HN-(1-12)	[SEQ ID NO:80]
[Ser^1, Lys^7]HN-(1-12)-amide	[SEQ ID NO:80]
[Ser^1, Lys^7]HN-(1-10)	[SEQ ID NO:81]
[Ser^1, Lys^7]HN-(1-10)-amide	[SEQ ID NO:81]
[Lys^2]HN-(2-15)	[SEQ ID NO:82]
[Lys^2]HN-(2-15)-amide	[SEQ ID NO:82]
[Lys^2]HN-(2-12)	[SEQ ID NO:83]
[Lys^2]HN-(2-12)-amide	[SEQ ID NO:83]
[Lys^2]HN-(2-10)	[SEQ ID NO:84]
[Lys^2]HN-(2-10)-amide	[SEQ ID NO:84]
[Lys^7]HN-(2-15)	[SEQ ID NO:85]

[Lys^{7}]HN-(2-15)-amide	[SEQ ID NO:85]
[Lys^{7}]HN-(2-12)	[SEQ ID NO:86]
[Lys^{7}]HN-(2-12)-amide	[SEQ ID NO:86]
[Lys^{7}]HN-(2-10)	[SEQ ID NO:87]
[Lys^{7}]HN-(2-10)-amide	[SEQ ID NO:87]
HN-(3-15)	[SEQ ID NO:88]
HN-(3-15)-amide	[SEQ ID NO:88]
HN-(3-12)	[SEQ ID NO:89]
HN-(3-12)-amide	[SEQ ID NO:89]
HN-(3-10)	[SEQ ID NO:90]
HN-(3-10)-amide	[SEQ ID NO:90]
HN-(4-15)	[SEQ ID NO:91]
HN-(4-15)-amide	[SEQ ID NO:91]
HN-(4-12)	[SEQ ID NO:92]
HN-(4-12)-amide	[SEQ ID NO:92]
HN-(4-10)	[SEQ ID NO:93]
HN-(4-10)-amide	[SEQ ID NO:93]
HN-(5-15)	[SEQ ID NO:94]
HN-(5-15)-amide	[SEQ ID NO:94]
HN-(5-12)	[SEQ ID NO:95]
HN-(5-12)-amide	[SEQ ID NO:95]
HN-(5-10)	[SEQ ID NO:96]
HN-(5-10)-amide	[SEQ ID NO:96]
HN-(6-15)	[SEQ ID NO:97]
HN-(6-15)-amide	[SEQ ID NO:97]
HN-(6-12)	[SEQ ID NO:98]
HN-(6-12)-amide	[SEQ ID NO:98]
HN-(6-10)	[SEQ ID NO:99]
HN-(6-10)-amide	[SEQ ID NO:99]
[Ser^{1}]HN-Cys^{16}	[SEQ ID NO:100]
[Ser^{1}]HN-Cys^{16}-amide	[SEQ ID NO:100]
[Thr^{1}]HN-Cys^{16}	[SEQ ID NO:101]
[Thr^{1}]HN-Cys^{16}-amide	[SEQ ID NO:101]
[Gly^{1}]HN-Cys^{16}	[SEQ ID NO:102]
[Gly^{1}]HN-Cys^{16}-amide	[SEQ ID NO:102]
[Leu^{1}]HN-Cys^{16}	[SEQ ID NO:103]
[Leu^{1}]HN-Cys^{16}-amide	[SEQ ID NO:103]

[Ala^1]HN-Cys^{16} [SEQ ID NO:104]
[Ala^1]HN-Cys^{16}-amide [SEQ ID NO:104]
[Val^1]HN-Cys^{16} [SEQ ID NO:105]
[Val^1]HN-Cys^{16}-amide [SEQ ID NO:105]
[Ile^1]HN-Cys^{16} [SEQ ID NO:106]
[Ile^1]HN-Cys^{16}-amide [SEQ ID NO:106]
[Phe^1]HN-Cys^{16} [SEQ ID NO:107]
[Phe^1]HN-Cys^{16}-amide [SEQ ID NO:107]
[Tyr^1]HN-Cys^{16} [SEQ ID NO:108]
[Tyr^1]HN-Cys^{16}-amide [SEQ ID NO:108]
HN-Cys^{16} [SEQ ID NO:109]
HN-Cys^{16}-amide [SEQ ID NO:109]
[Cys^1]HN-Cys^{16} [SEQ ID NO:110]
[Cys^1]HN-Cys^{16}-amide [SEQ ID NO:110]
[Val^1, Val^2, Lys^7]HN [SEQ ID NO:111] and
[Val^1, Val^2, Lys^7]HN-amide [SEQ ID NO:111].

54. A peptide according to claim 2, said peptide being selected from the group consisting of:

HN-(2-15) [SEQ ID NO: 112]
HN-(2-15)-amide [SEQ ID NO: 112]
HN-(7-15) [SEQ ID NO: 113]
HN-(7-15)-amide [SEQ ID NO: 113]
HN-(8-15) [SEQ ID NO: 114]
HN-(8-15)-amide [SEQ ID NO: 114]
[Ser^1]HN-(1-14) [SEQ ID NO: 115]
[Ser^1]HN-(1-14)-amide [SEQ ID NO: 115]
[Ser^1]HN-(1-13) [SEQ ID NO: 116]
[Ser^1]HN-(1-13)-amide [SEQ ID NO: 116]
[Ser^1, His^2]HN [SEQ ID NO: 117]
[Ser^1, His^2]HN-amide [SEQ ID NO: 117]
[Gln^1]HN [SEQ ID NO: 118]
[Gln^1]HN-amide [SEQ ID NO: 118]
[Ser^1, Ala^2]HN [SEQ ID NO: 119]
[Ser^1, Ala^2]HN-amide [SEQ ID NO: 119]
[Ser^1, Ser^2]HN [SEQ ID NO: 120]
[Ser^1, Ser^2]HN-amide [SEQ ID NO: 120]

[Glu^1]HN	[SEQ ID NO: 121]
[Glu^1]HN-amide	[SEQ ID NO: 121]
[Ser^1]HN-Gly^{16}	[SEQ ID NO: 122]
[Ser^1]HN-Gly^{16}-amide	[SEQ ID NO: 122]
[Ser^1, His^2]HN	[SEQ ID NO: 123]
[Ser^1, His^2]HN-amide	[SEQ ID NO: 123]
HN-(2-10)	[SEQ ID NO: 124]
HN-(2-10)-amide	[SEQ ID NO: 124]
[Arg^2]HN	[SEQ ID NO: 125]
[Arg^2]HN-amide	[SEQ ID NO: 125]
[Lys^2]HN	[SEQ ID NO: 126]
[Lys^2]HN-amide	[SEQ ID NO: 126]
[pGlu^1]HN	[SEQ ID NO: 127]
[pGlu^1]HN-amide	[SEQ ID NO: 127]

55. The use of a peptide as a blocker or potentiator of the NMDA and/or AMPA receptors for glutamate (Glu) in mammals, said peptide being histogranin [SEQ ID NO:1] or a histogranin analog having a structure according to the following general formula:

$$H\text{-}R_1\text{-Gln-Gly-Arg-}R_2\text{-CO-}R_3$$

wherein:

R_1 represents one structure selected from the group consisting of:

X-Asn-Tyr-Ala-Leu-Lys-Gly (X being an hydroxyl-containing amino acid [Ser, Thr]);

Y-Asn-Tyr-Ala-Leu-Lys-Gly (Y being a hydrocarbon side chain-containing amino acid [Gly, Ala, Leu,Val, Ile]);

Z-Asn-Tyr-Ala-Leu-Lys-Gly (Z being an aromatic amino acid [Phe, Tyr]);

W-Asn-Tyr-Ala-Leu-Lys-Gly (W being a sulfur-containing amino acid [Met, Cys]);

A-Asn-Tyr-Ala-Leu-Lys-Gly; Ser-A-Tyr-Ala-Leu-Lys-Gly; Ser-Asn-Tyr-Ala-Leu-Lys-A; A-Tyr-Ala-Leu-Lys-Gly; Asn-Tyr-Ala-Leu-Lys-A; Tyr-Ala-Leu-A-Gly; Ala-Leu-A-Gly; Leu-A-Gly; A-Gly; and Val-Val-Tyr-Ala-Leu-Lys-A (A being a basic amino acid [Arg, Lys]);

R_2 represents one structure selected from the group consisting of:

a single covalent bond;

Thr-Leu;

Thr-Leu-Tyr-Gly-Phe;

Thr-Leu-Tyr-Gly-Phe-Cys; and

Thr-Leu-Tyr-Gly-Phe-Gly-Gly;

and R_3 represents a radical selected from the group consisting of -OH and $-NH_2$.

56. The use of a peptide as a blocker or enhancer of the immune system in mammals, said peptide being histogranin [SEQ ID NO:1] or a histogranin analog having a structure according to the following general formula:

$$H-R_1-Gln-Gly-Arg-R_2-CO-R_3$$

wherein:

R_1 represents one structure selected from the group consisting of:

X-Asn-Tyr-Ala-Leu-Lys-Gly (X being an hydroxyl-containing amino acid [Ser, Thr]);

Y-Asn-Tyr-Ala-Leu-Lys-Gly (Y being a hydrocarbon side chain-containing amino acid [Gly, Ala, Leu, Val, Ile]);

Z-Asn-Tyr-Ala-Leu-Lys-Gly (Z being an aromatic amino acid [Phe, Tyr]);

W-Asn-Tyr-Ala-Leu-Lys-Gly (W being a sulfur-containing amino acid [Met, Cys]);

A-Asn-Tyr-Ala-Leu-Lys-Gly; Ser-A-Tyr-Ala-Leu-Lys-Gly; Ser-Asn-Tyr-Ala-Leu-Lys-A; A-Tyr-Ala-Leu-Lys-Gly; Asn-Tyr-Ala-Leu-Lys-A; Tyr-Ala-Leu-A-Gly; Ala-Leu-A-Gly; Leu-A-Gly; A-Gly; and Val-Val-Tyr-Ala-Leu-Lys-A (A being a basic amino acid [Arg, Lys]);

R_2 represents one structure selected from the group consisting of:

a single covalent bond;

Thr-Leu;

Thr-Leu-Tyr-Gly-Phe;

Thr-Leu-Tyr-Gly-Phe-Cys; and

Chr-Leu-Tyr-Gly-Phe-Gly-Gly;

and R_3 represents a radical selected from the group consisting of -OH and $-NH_2$.

Fig. 1.

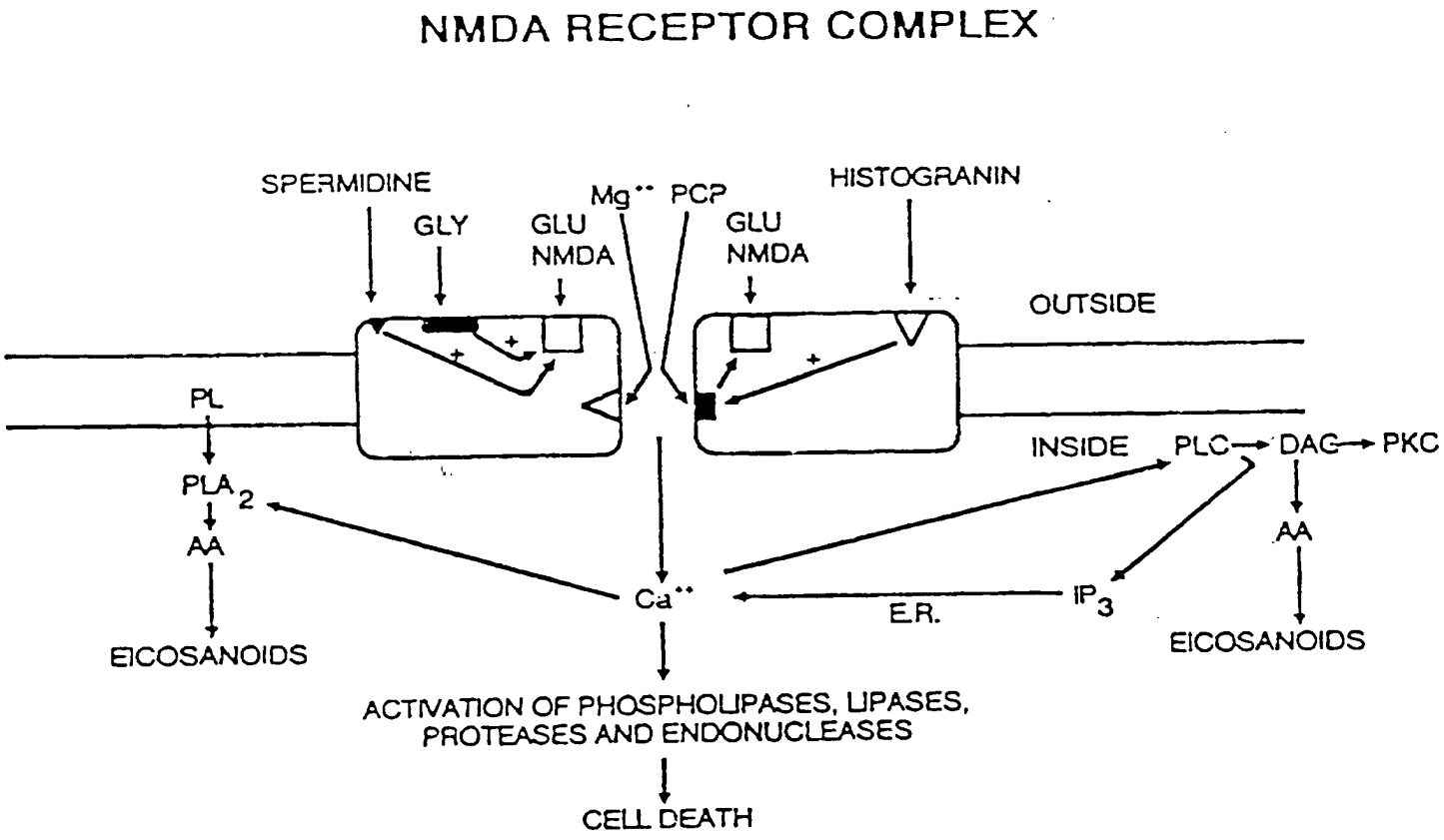

Fig. 2

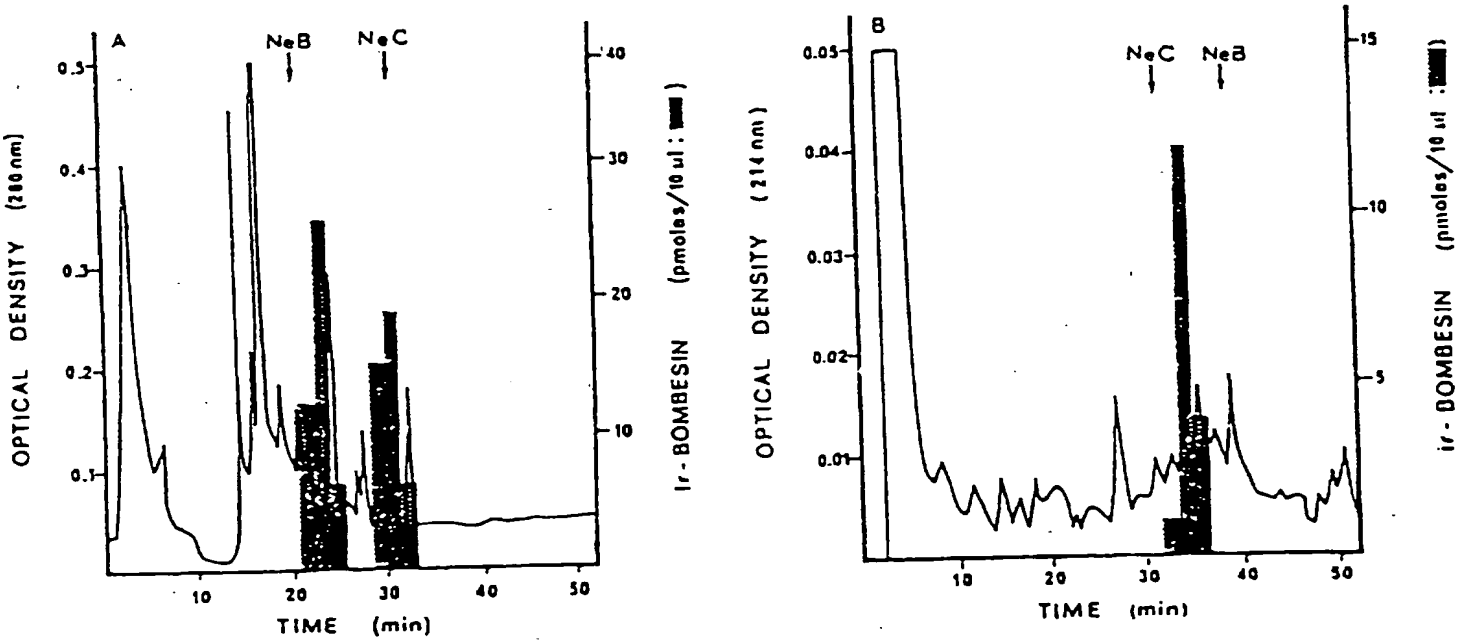
A
NeB
NeC
OPTICAL DENSITY (280 nm)
Ir-BOMBESIN (pmoles/10 µl : ■)
TIME (min)
B
NeC
NeB
OPTICAL DENSITY (214 nm)
Ir-BOMBESIN (pmoles/10 µl : ■)
TIME (min)

Fig. 3

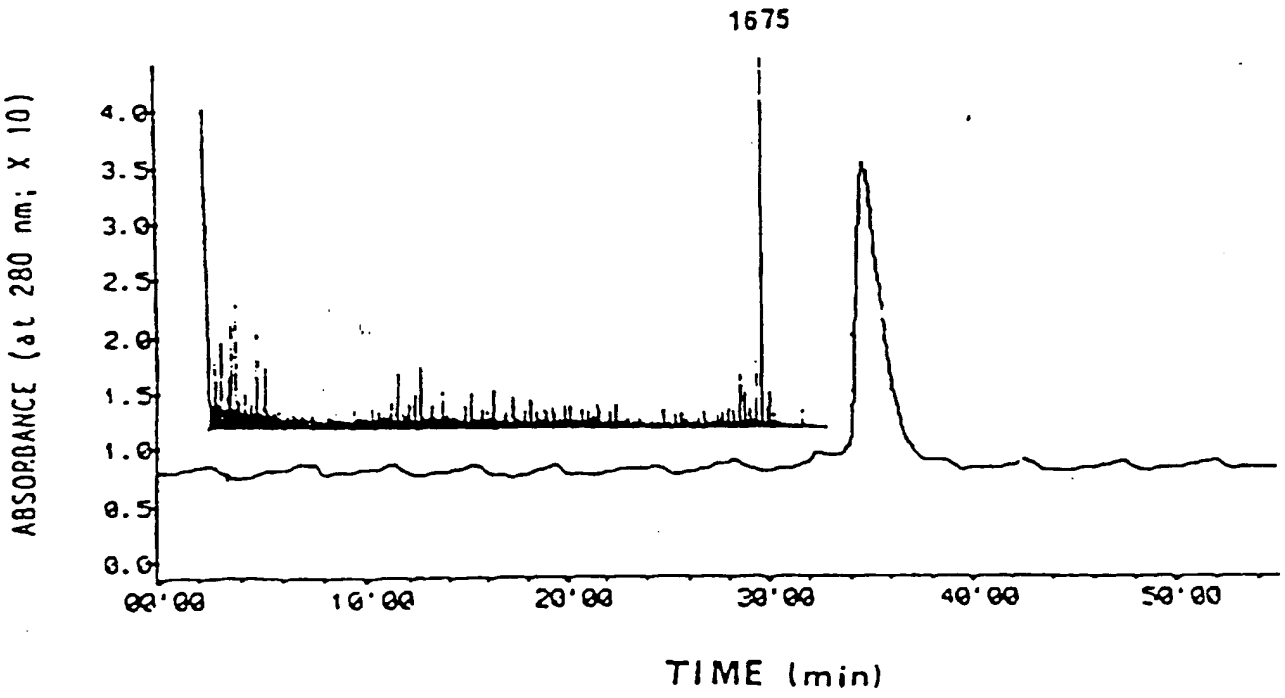

Fig. 4

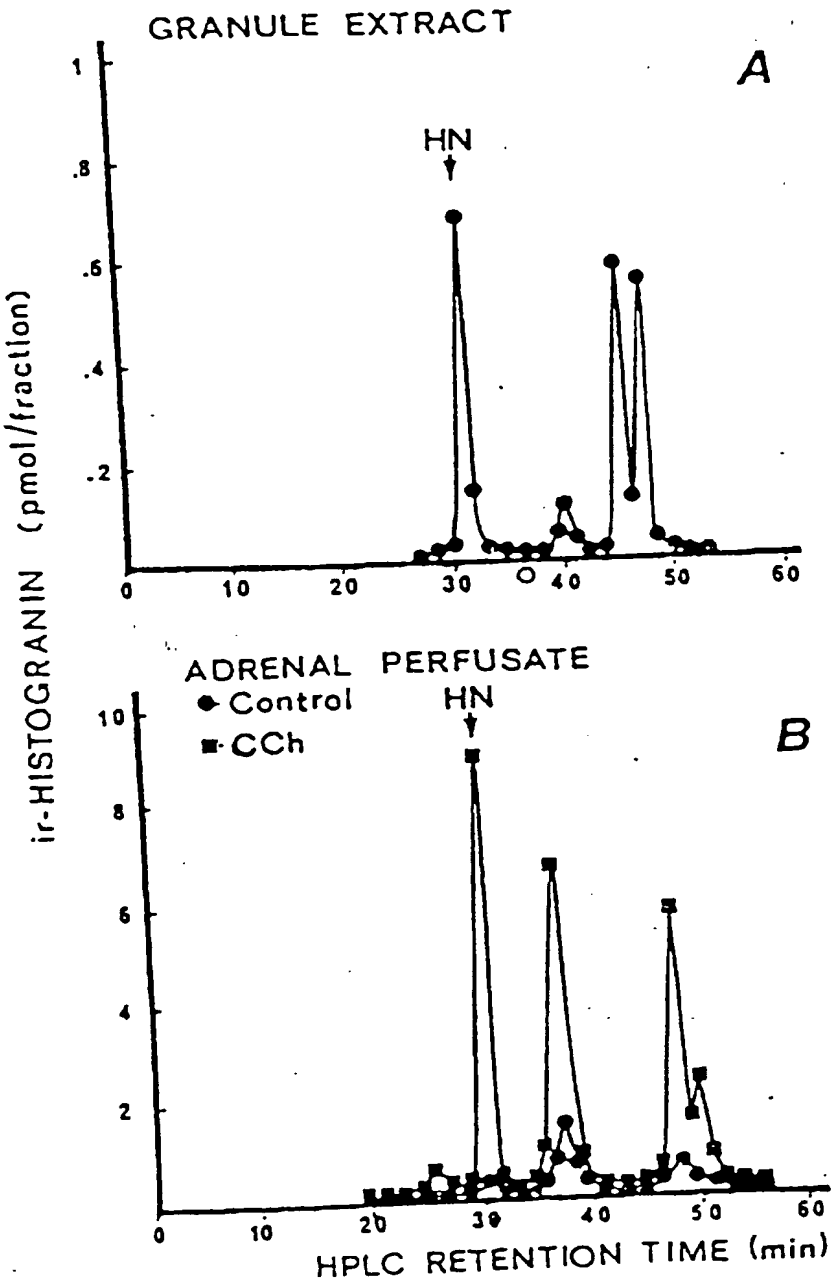
GRANULE EXTRACT
A
HN
ir-HISTOGRANIN (pmol/fraction)
1
.8
.6
.4
.2
0
10
20
30
40
50
60
ADRENAL PERFUSATE
Control
CCh
HN
B
10
8
6
4
2
0
10
20
30
40
50
60
HPLC RETENTION TIME (min)

Fig. 5

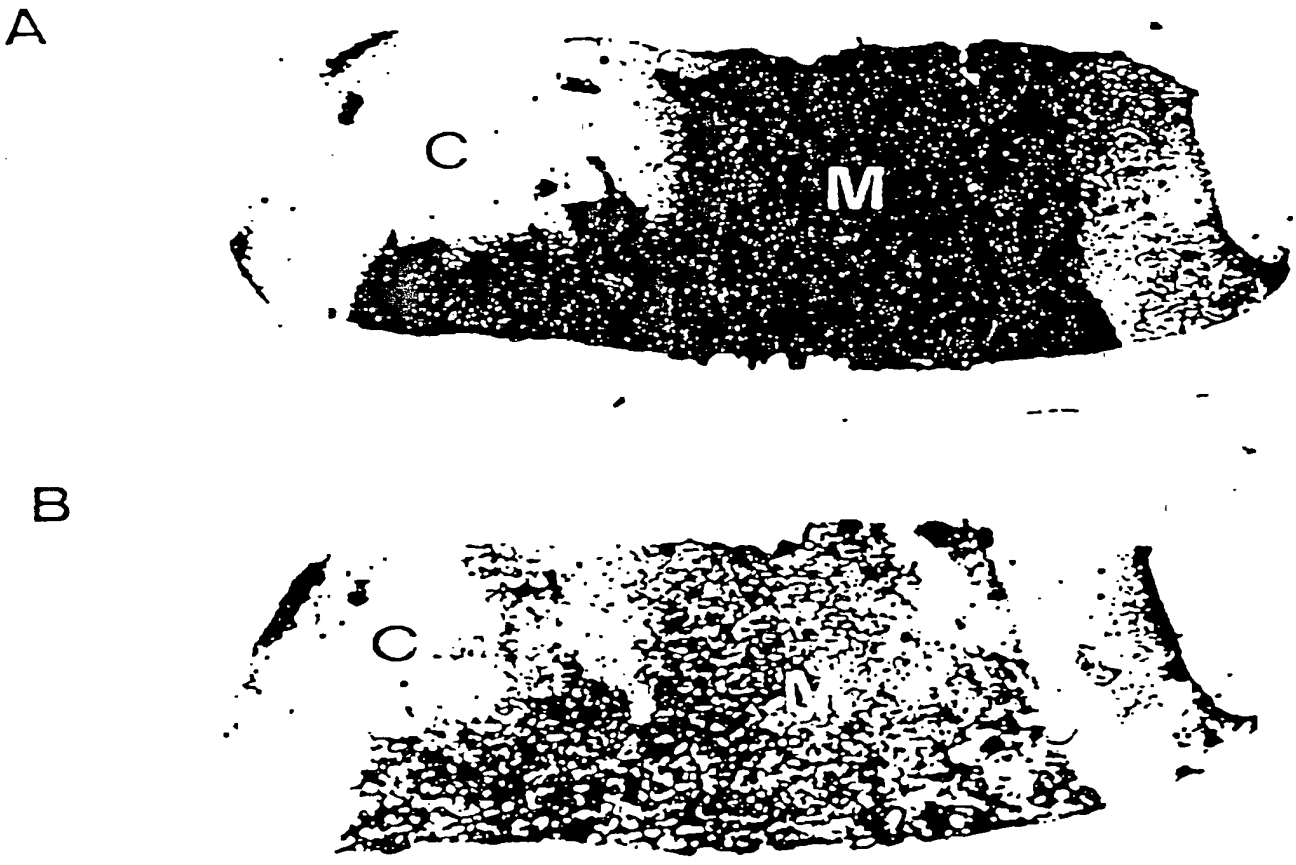

Fig. 6

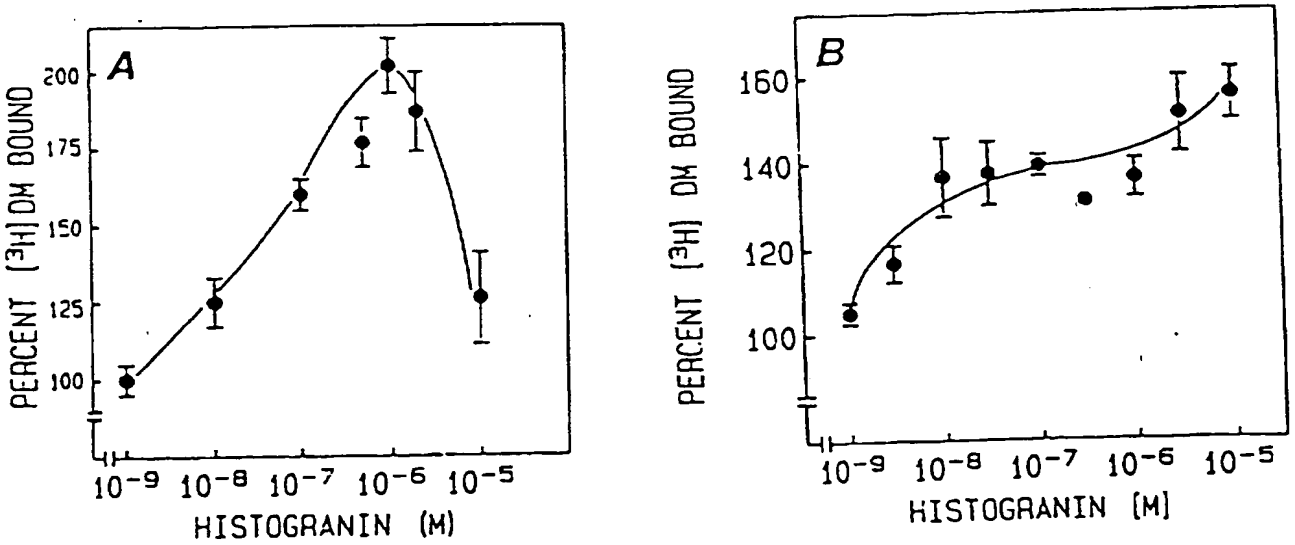

Fig. 7

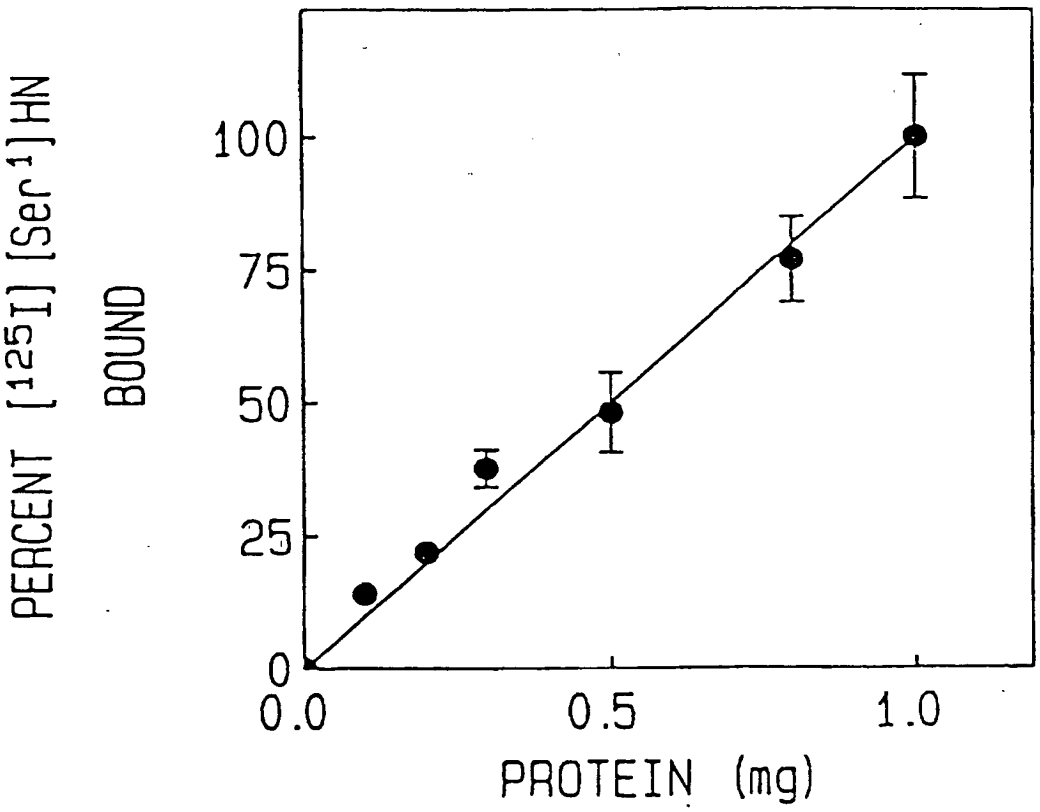

Fig. 8

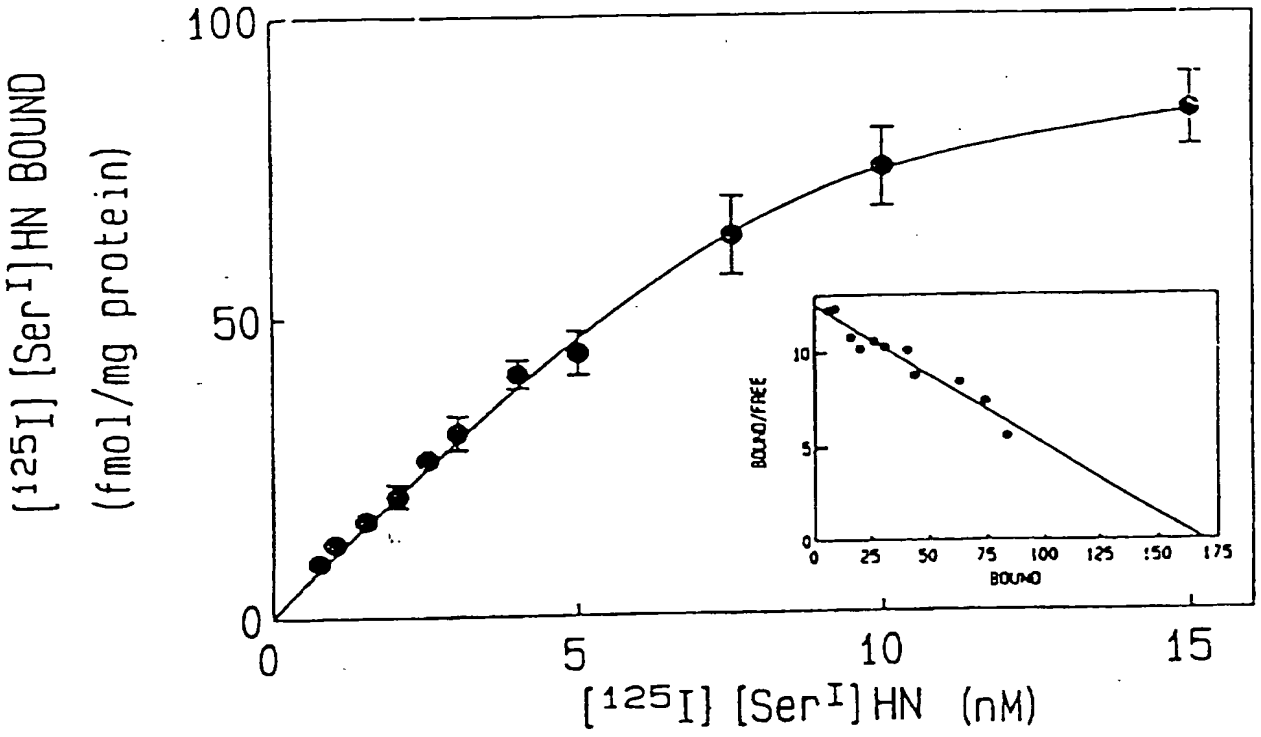

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Fig. 9

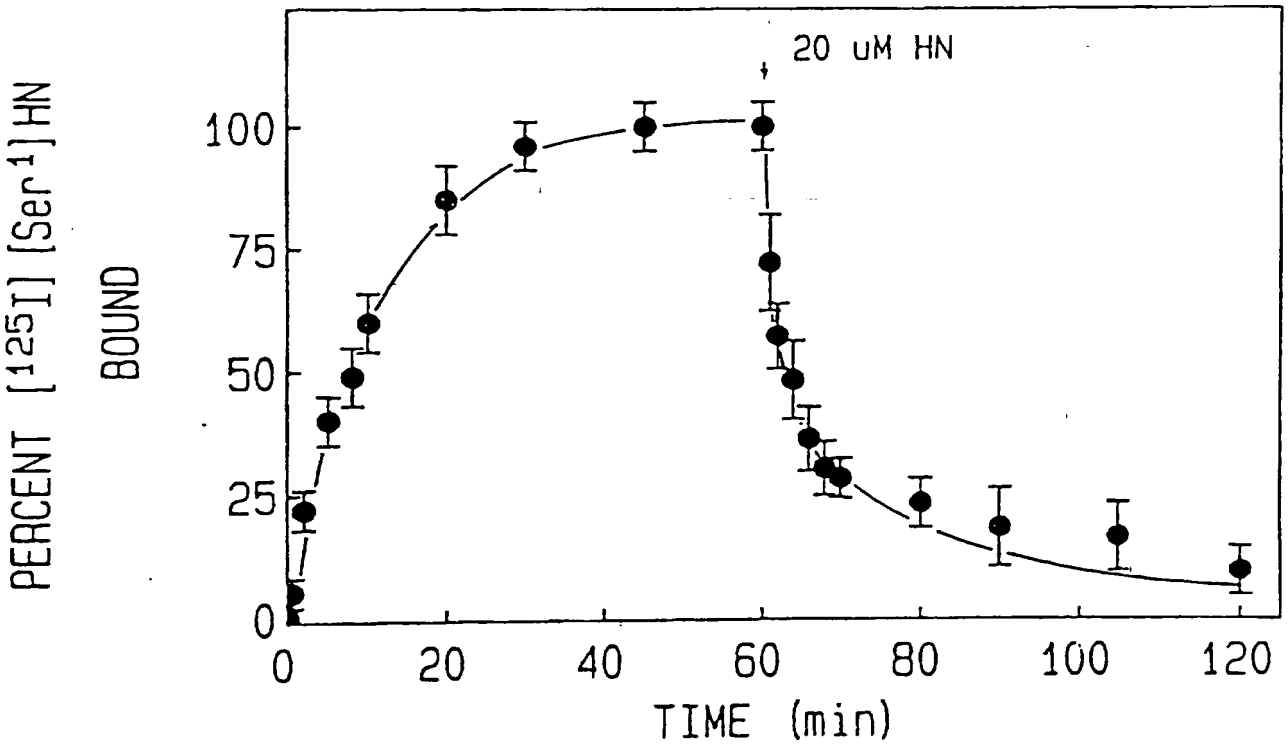


Fig. 10

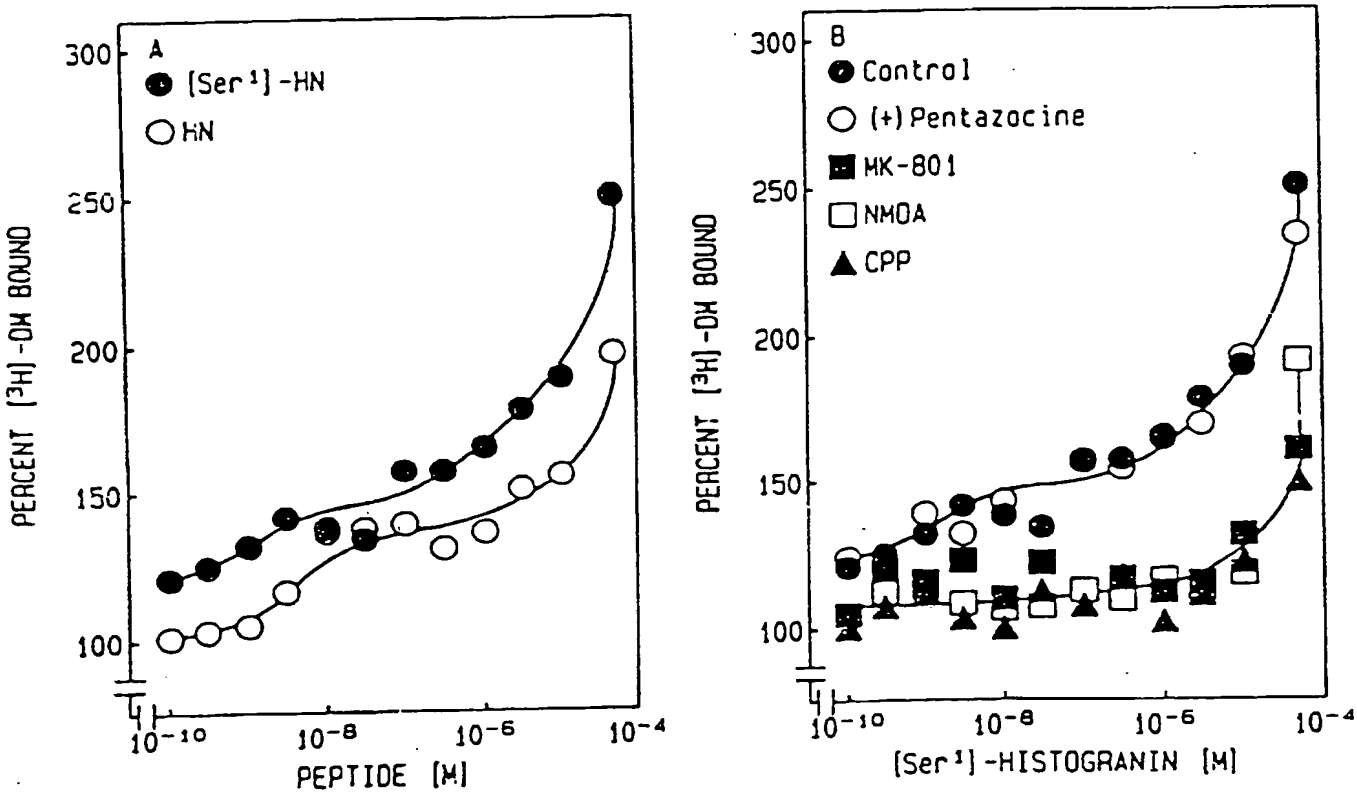


Fig. 11

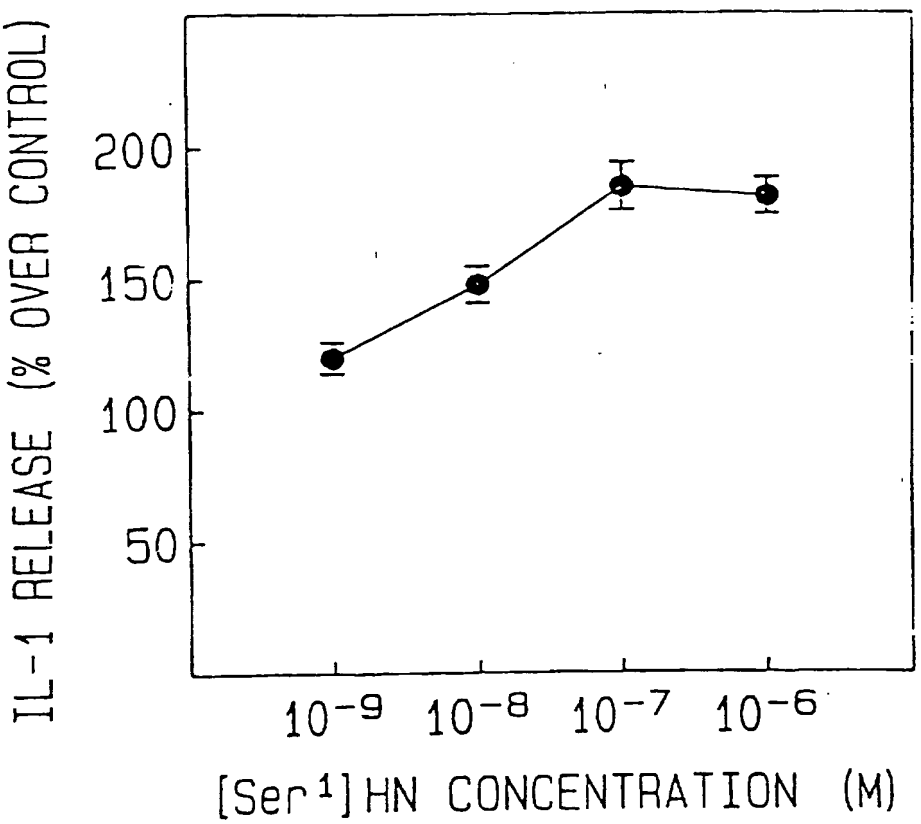
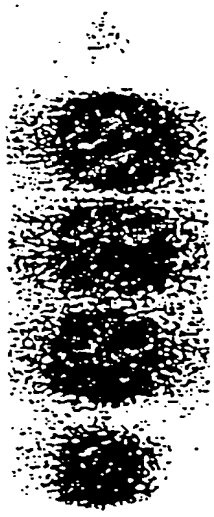


Fig. 12



IL-1α mRNA

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Fig. 13